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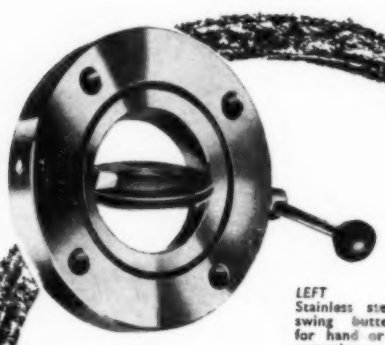

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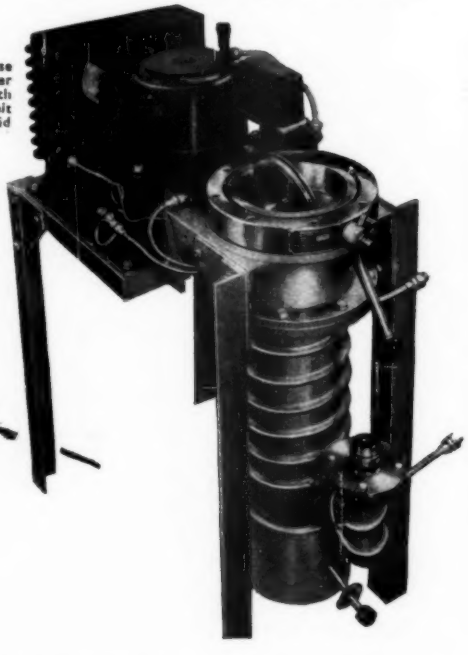

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No. 4757 SATURDAY, DECEMBER 31, 1960 Vol. 188

CONTENTS

	Page
Colleges of Advanced Technology	1137
Geographical Distribution of European Birds. By Dr. D. Lack, F.R.S.	1139
Sea Birds. By Miss Frances Pitt	1139
Crystallography Condensed. By Dr. M. G. Bown	1139
Geology in the United States. By Prof. C. F. Davidson, O.B.E.	1140
Coal Science: an Indian Venture. By Dr. I. G. C. Dryden	1140
Pests and their Control. By F. H. Jacob	1141
Growing Points in Plants. By Dr. F. A. L. Clowes	1141
Flora of Tropical East Africa. By J. P. M. Brenan	1142
Low-temperature Forms of Ice as studied by X-Ray Diffraction. By L. G. Dowell and A. P. Rinfret	1144
The Cole Library of Zoology and Early Medicine, University of Reading	1148
Obituaries:	
Prof. W. N. Thomas, C.B.E. By Prof. C. Gurney	1151
Dr. Pierre A. Chappuis. By Dr. Isabella Gordon	1151
Dr. Gertrude L. Elles, M.B.E. By Prof. O. M. B. Bulman, F.R.S.	1152
News and Views	1152
Organization of Scientific Research in Latin America. By Prof. B. A. Houssay, For.Mem.R.S.	1157
International Operational Research. By Stafford Beer	1158
Biological Structure and Function. By Prof. T. W. Goodwin	1160
Powders in Industry. By Dr. S. H. Bell	1161
Composition of Milk in the United Kingdom	1162
A Theory of the Mechanism of Action of Creatine Phosphokinase. By Dr. B. R. Rabin and D. C. Watts	1163
A Possible Specific Chromosome Abnormality in Human Chronic Myeloid Leukæmia. By A. G. Baikie, W. M. Court-Brown, Karin E. Buckton, D. G. Harnden, Patricia A. Jacobs and Ishbel M. Tough	1165
Blue Wildebeest and the Ætiological Agent of Bovine Malignant Catarrhal Fever. By W. Plowright, R. D. Ferris and G. R. Scott	1167
Role of Cereal Fat in the Production of Nutritional Disease in Pigs. By B. Thafvelin	1169
The Biospecies in Entomology. By M. F. Claridge	1172
Some Oxidative and Hydroxylative Actions of Quartz: their Possible Relationship to the Development of Silicosis. By L. W. Marasas and Dr. J. S. Harington	1173
Recent Scientific and Technical Books	Supp. vi

(Continued overleaf)

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LETTERS TO THE EDITORS

Page

ASTRONOMY

Bode's Law and the Solar System.—Dr. M. M. Woolfson 1175
Spheroidal Oscillations of the Moon.—Bruce A. Bolt 1176

PHYSICS

Cæsium-137 Fall-out over Norway since September 1956.—T. Hvinden, D. Hveding, A. Lillegraven and S. H. Small 1177
New Delayed-Neutron Precursors.—G. Rudstam, Å. Svanheden and Prof. A. C. Pappas . . 1178
Natural Occurrence of Samarium-146.—Ronald D. Macfarlane 1180
Radiation-induced Defects in Lead Silicate Glass.—R. S. Barker, D. A. Richardson, E. A. G. McConkey and R. E. Yeadon 1181

ENGINEERING

Beneficial Effects of Reagents in Solution on Wet Crushing of Rock.—S. K. Ghosh, C. C. Harris and Dr. A. Jowett 1182
Hydrogen Diffusion in Water-accelerated Rolling Surface Fatigue.—Dr. L. Grunberg, D. T. Jamieson, D. Scott and R. A. Lloyd . . 1182

METALLURGY

Fatty Acids and Adhesion of Electrodeposited Metals.—Dr. P. A. Brook 1183
Fatigue Straining of Copper Whisker.—Dr. E. Eisner 1183

RADIOCHEMISTRY

Effect of γ-Radiation on Nisin.—F. J. Ley and R. H. Hall 1184

CHEMISTRY

Electron Paramagnetic Resonance Studies of Stable Free Radicals in Lignins and Humic Acids.—Dr. R. W. Rex 1185
Surface Renewal and Molecular Diffusion in Interphase Mass Transfer.—N. G. Maroudas and Dr. H. Sawistowski 1186
Growth and Adhesion of Oxide in Furnace Deposits.—Miss J. Steel 1187
New Data for the Calcium Silicate, 'Phase Z'.—Dr. D. M. Roy, Dr. J. A. Gard, A. W. Nicol and Dr. H. F. W. Taylor 1187

BIOCHEMISTRY

Different Action of 2 : 4-Dinitrophenol on the Oxidation of Exogenous and Endogenous Substrates by Baker's Yeast.—Prof. A. O. M. Stoppani, Dr. E. H. Ramos and I. Widuczynski 1188
Mechanism of Activation and Reduction of α-Aminoadipic Acid by Yeast Enzyme.—S. Sagisaka and Prof. K. Shimura 1189
Paper Chromatography and Paper Electrorheophoresis of Tuberculoproteins.—T. P. Dekker . 1191
Evidence for the Existence of Two Corticotrophin-releasing Factors, α and β.—Prof. A. V. Schally, R. N. Andersen, Prof. H. S. Lipscomb, J. M. Long and Prof. Roger Guillemin . . . 1192
Spontaneous Plasma Kinin Formation in Human Plasma collected during Labour.—Dr. Desirée A. J. Armstrong and Dr. J. W. Stewart . . 1193
Isolation of Hyaluronate from Human Synovial Fluid by Zone Electrophoresis.—Dr. David Hamerman and John Sandson 1194

Page

Inhibitor of the Cytochrome Oxidase of Unfertilized Sea Urchin Eggs.—Rachele Maggio, Franca Ajello and Prof. Alberto Monroy 1195

PHYSIOLOGY

Thyroid Function and Oxidative Phosphorylation in Cold-exposed Rats.—Dr. Sylva Panagos Lianides and Dr. Robert E. Beyer 1196
Abolition by 2 : 4-Dinitrophenol of the Protective Effect of Succinate and Adenine Nucleotides on the Onset of the Thyroxine-induced Swelling of Liver Mitochondria.—Dr. P. Emmelot . . 1197
Accelerated Recovery from Reserpine Depression by Monoamine Oxidase Inhibitors.—Larry Stein and Oakley S. Ray 1199
Electrophoretic Mobilities of Normal and Regenerating Liver Cells.—S. Ben-Or, S. Eisenberg and Dr. F. Doljanski 1200

HÆMATOLOGY

Separation of Reticulocytes by means of Multi-Layer Centrifugation.—Prof. Eiichi Kimura, Tatsuo Suzuki and Yoshihiro Kinoshita . . 1201
Human Embryo Hæmoglobin.—Dr. Harry Zilliacus 1202

PATHOLOGY

Leucocytic Emigration from Inflamed Capillaries.—Dr. J. R. Williamson and Dr. J. W. Grisham 1203
Blood Corticotrophin in Cushing's Syndrome.—Beryl M. A. Davies, A. R. Currie and T. Symington 1203

RADIOBIOLOGY

A Sexual Difference in the Concentration of Iodine-131 by the Submaxillary Gland of Mice.—J. L. Llach, J. H. Tramezzani and J. R. Cordero Funes 1204
Uptake of Magnesium-28 by the Skeleton of a Sheep.—Alexander C. Field 1205

BIOLOGY

Effect of Chelating Agents on Plasmodium Formation by the True Slime Mould, *Didymium nigripes*.—Prof. Norman S. Kerr . . . 1206
Effect of Extracts from Fucoids in promoting Settlement of Epiphytic Polyzoa.—Dr. D. J. Crisp and G. B. Williams 1206
Sex Hormones as a Possible Factor influencing the Level of Parasitization in Frogs.—Dr. E. Lees and L. Bass 1207
Mobility of Barnacles.—Dr. D. J. Crisp . . 1208
Mechanism of Change in Permeability in Living Membranes.—Dr. A. M. Shanes 1209
Colchi-Autotetraploidy in *Adiantum capillus-veneris*.—S. C. Verma and Dr. D. S. Loyal . 1210

MICROBIOLOGY

Alleged Transformation of Yeast.—G. Harris and C. C. Thompson 1212

VIROLOGY

Plaque Formation by an Orphan Virus of Fowls.—I. A. Macpherson 1213
Effect of Ultrasonic Vibration on the Hæmagglutinating Activity of Some Para-influenza Viruses.—Svante Hermodsson 1214

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COLLEGES OF ADVANCED TECHNOLOGY

THE Advisory Council on Scientific Policy in its last report expressed the opinion that the development of colleges of advanced technology in Britain and the expansion of the smaller or medium-sized universities, which have hitherto included only faculties of arts or pure science, afforded an opportunity unlikely to recur for promoting studies in fields where there is little or no tradition of research and its application, and where there is little interaction between the universities and the industries which are the customers for the resulting technological skills. In the subsequent debate on the Council's report in the House of Lords, the question of research in the colleges of technology was scarcely mentioned, but in speaking on educational policy in the House of Commons on November 7, the Minister of Education, Sir David Eccles, after referring to the steady growth of postgraduate work in these colleges which the Government proposes to encourage as much as possible, said that he is very keen that these colleges should vigorously develop applied research.

Sir David expressed the view that the colleges of advanced technology are excellently placed to do so, because of their close links with industry; and those links would undoubtedly assist in the selection of projects for research that are really worth while. This appears to be what the Advisory Council on Scientific Policy also has in mind, but it is not here that the real difficulties in encouraging research in technical colleges lie. They are to be found in relation to staff, to problems of administration and status, and to a certain extent in finance. These difficulties, however, were not considered in the debate, though Mr. A. Albu doubtless had them in mind when he urged that the colleges of advanced technology should have independent governing bodies and should not come under the local authorities.

How small is the comparative contribution from the colleges of advanced technology may be seen from some figures quoted by Dr. D. C. Freshwater in *Technology*. Against an estimated 6,000 postgraduate workers in science and technology in the universities, there are only some 200 scientists and technologists on full-time postgraduate research in these colleges, of whom 40 are financed by Government grants, 20 by awards through the Department of Scientific and Industrial Research and 140 by the local education authorities. Of these 200, some 120 are in colleges in the London area and only about 80 in the remainder of the country, including those industrial areas of the Midlands and the North where it might be expected that close contact with local industry would be most conducive to stimulating exactly the type of technological research that the Advisory Council on Scientific Policy suggested in its last report.

Any advantage that the colleges of technology may enjoy through closer relations with local industry in the selection of projects for research, and possibly for the conduct of such research, can be more than offset if the conditions these colleges offer are not such as to attract staff of the requisite quality or to retain competent postgraduate students. The function of the colleges of technology in advancing knowledge, especially knowledge of value to industry, is still insufficiently recognized, and not all local authorities responsible for such colleges appreciate that research can make an important contribution in the teaching of advanced students, the vitality it imparts to the instruction being as important here as in a university. There are still local education authorities which do not acknowledge research as a normal and indeed an important function of the technical colleges. In consequence, there is no general pattern to the scope and practice of research in progress at the present time; and even where it is formally recognized, lack of facilities and equipment is an obstacle to the postgraduate research student, and the lecturer may find difficulties in giving time to research as distinct from teaching.

In a recent article in the *Journal of the Royal Institute of Chemistry* (84, 235; 1960), Dr. D. E. Chisman describes the position more particularly with regard to chemical research, which is believed to be in progress to some extent in at least forty colleges, or about half the technical colleges recognized by the Institute for training candidates for the graduate membership examination. In two of these there are more than fifty research workers in the chemistry department; but this includes part-time and full-time workers, both for the teaching staff and research students. In a further five colleges there are between 25 and 50 research workers; but in most of the colleges there are fewer than ten research workers, sometimes just one or two members of the staff who are endeavouring to complete research projects started elsewhere. Altogether, Dr. Chisman suggests that in the technical colleges in 1959–60 there were more than 300 chemistry research projects under way.

Again, the facilities available for research vary widely. Sometimes the only space available is in a corner or at one end of a teaching laboratory. Elsewhere small rooms have been partitioned off from large laboratories; but in a few colleges the facilities have been appropriately designed, and an area up to 3,500 sq. ft. is available, though the usual area specifically available where a research laboratory is provided seems to be about 1,000 sq. ft. Especially in the smaller colleges, research in chemistry is at present undertaken only by members of the teaching staff, and their research interests have to be integrated with their teaching duties. If the latter are particularly onerous, the time for research is naturally curtailed, and the research project at best is protracted, if indeed it is ever completed.

To help members of staff who have genuine research projects in train, many local education authorities are prepared to allow some time for research in lieu of teaching duties, and according to Dr. Chisman this usually amounts to 3–6 hr. a week, although for a reader or principal lecturer it may be 12 hr. per week. Not all local authorities, however, appreciate the desirability of allowing teaching staff time for research, and Sir David Eccles's observation in the House of Commons should induce some improvement here, though it is reasonable to expect that the teaching staff concerned should display some initiative and enthusiasm, and disclose the nature of their projects. The brochure issued by the London and Home Counties Regional Advisory Council for Higher Technological Education on research in progress in technical colleges of the region, for, or in collaboration with, industry, Government departments and research associations indicates the range and value of such projects even in 1956.

One disappointing feature of the present situation to which Dr. Chisman refers is the comparatively small number of students of research calibre, to whom research studentships in science and technology have been awarded through the Department of Scientific and Industrial Research, who elect to receive their training in technical colleges. There are at present more than 2,000 such awards held in universities and technical colleges in Britain, and it is expected that about a further 1,000 awards will be made this year. Less than 2 per cent of such research studentships in 1958 were for tenure in technical colleges, mostly in chemistry.

Some, but not all, research in colleges of technology is undertaken for the purpose of obtaining a higher degree or equivalent qualification, and the new award of membership of the College of Technology, introduced by the National Council for Technological Awards, which will normally be made for a programme of work (not necessarily research) carried out jointly in industry and at a college, should assist in removing any difficulties which research workers at the colleges of technology may experience in obtaining a higher qualification. While the College of Technologists will expect a high standard of candidates, the award will not be restricted to holders of the diploma in technology. Here there is, of course, a time factor: the award has to establish its merits, and this might perhaps be the easier if there were less readiness to blur the distinction between colleges of technology and the technical colleges. To say the least, it is debatable how far postgraduate work is really practicable in an institution which is not devoted to instruction at university-level.

Dr. Chisman expresses the opinion that there are probably in the colleges of technology more *ad hoc* short-term research projects in progress at any given time than research designed specifically for a higher degree or equivalent. Local industrial needs often lead to the initiation of short-term special research problems, thus effecting a valuable link between industry and technical education. Moreover, industry can, and does, assist by giving expensive equipment, and some colleges have thus been able to develop useful specialized research facilities. This is obviously a step towards the co-operation in the attack on major technological problems which the Advisory Council on Scientific Policy had in mind in its last report. If the major colleges of technology are to develop schools of research in particular technological fields, it will be just as essential that the immensely expensive equipment, which technological research no less than scientific research requires, shall be used on an agreed co-operative basis—perhaps between such colleges themselves, or between a particular college or group of colleges and industry—as it has now become for the universities.

Developments along such lines are likely to provide one of the surest ways of winning the prestige that will enable the colleges to attract—and keep—all the research workers they are able to accommodate. They will also help to solve the problem of status; but it must be realized that the question of status and autonomy cannot be left to chance. They must be tackled simultaneously and urgently. Research workers of the requisite calibre will not be drawn to the colleges of technology unless they are assured of a status comparable with that which they would enjoy in a university institution, and have the freedom to teach and investigate which it is a primary function of academic autonomy to ensure. The independence of the colleges of technology is a problem which needs urgent consideration, and local interest should not be allowed to overrule the national interest. Given goodwill and understanding and sense, the reconciliation of a live interest in local institutions with the larger national concern should prove no more difficult than in the creation of new university institutions.

One final aspect of research noted by Dr. Chisman might well make a valuable contribution to this problem, as well as to the prestige of the colleges. Dr. Chisman refers to the need for investigations into methods of presenting the subject-matter used by lecturers in the technical colleges. As he rightly points out, the rapid developments of the past few years in the understanding of many phenomena through modern theories of atomic and sub-atomic structure have spurred the abler teachers to re-appraise their systems of teaching and to introduce substantial modifications, leading to more logical and co-ordinated presentation and to a more comprehensible and sound basis for further study. The whole content of university science courses as well as of school courses is under challenge and re-examination. There are also numerous ways in which standard experimental techniques may be improved and new demonstration techniques devised to illustrate theoretical topics. The colleges of technology may, in fact, enjoy more flexibility to take advantage of the considerable scope for such work, especially in the field of small-scale equipment; and in the measure to which they seize the opportunity, their prestige both as teaching and research institutions should be enhanced.

GEOGRAPHICAL DISTRIBUTION OF EUROPEAN BIRDS

Atlas of European Birds

By Prof. K. H. Voous. Pp. 284 (419 maps and 355 gravure plates). (Edinburgh and London: Thomas Nelson and Sons, Ltd., 1960.) 70*s*. net.

THIS splendidly produced volume fills a long-felt need in ornithology. A map is given showing the full breeding range of every species of bird that nests in Europe. For this purpose two basic maps have been used, one extending to the equator and the other covering the world, and so planned that one occupies half the space of the other, thus simplifying the arrangement. On each map the land is left uncoloured, the sea is shown in blue and the range of the bird is over-printed in red, which makes for extremely clear presentation. The author has used question-marks wherever there is doubt as to the range concerned.

The author's text is clear, concise and sound. He has in each case detailed the geographical range and habitat, and wherever these present problems of biological interest he has discussed them, so that the book is both a reliable summary of the known and a valuable guide to questions of zoo-geography and species-formation that would profitably repay further research. He has not discussed sub-specific variation except in a few cases where the allocation of species is difficult, but, while I think that it was fully justifiable to omit sub-specific names, I am sorry that he did not give a little more attention to the main trends of sub-specific variation. This is not, however, a serious omission. The author's treatment of the taxonomic limits of each species is very sound, and while it would be impossible to please all authorities, he has given his reasons where he differs from what may be found in other standard works. He has added short notes on food, nest-site and migration. Perhaps it is ungenerous to ask for more, but a second series of maps illustrating the range of each migratory species in winter is needed for proper evaluation of distributional problems, and such maps are not included.

The third section of the book is, in my view, irrelevant to the others. A black-and-white photograph is given of almost every species, providing a unique assemblage of fine photographs, even though many are already familiar from other bird-books. As a guide to identification, photographs can be misleading, particularly as there is no indication of the size of the bird. For example, in the double page featuring all the acrocephaline warblers, the smallest of all, to judge from the photographs, is the great reed warbler, which is in fact the largest of those shown. Anyway such photographs, being essentially bird portraits and not habitat pictures, are inappropriate to a bird atlas. There was, indeed, a strong case for two separate books, the first dealing with zoo-geography and the second with photographs, and omission of the latter from the atlas would, I imagine, have reduced its cost to less than half, and would have permitted printing on pages of half the present size, thus making it an even more valuable reference book than it is now. The photographs appeal to a different type of ornithologist and could have been issued separately with, perhaps, a brief description of each bird.

D. LACK

SEA BIRDS

Sea Birds

By Charles Vaucher. Translated by James Hogarth. Pp. 254 (255 photographs). (Edinburgh and London: Oliver and Boyd, Ltd., 1960.) 105*s*. net.

THIS is a sumptuous volume, beautifully produced, and its 255 photographs, 15 of which are in colour, are fine examples of the art of the camera. Charles Vaucher, their author, is to be congratulated on them. They include close-up bird portraits, snapshots of birds on the wing, some seascapes, among them an excellent picture of a four-mast sailing ship becalmed on a placid sea, and two or three shots of grey seals on the Farne Islands by way of contrast. Despite the seals, this is a bird book dealing with the birds to be found on or near the sea, particularly on the coasts of northern Europe, especially those of Britain and Scandinavia.

As indicated, this is primarily a picture book; but the literary matter is far from negligible or unimportant. The author amplifies the plates with chapters on the different species illustrated, their habits and habitats. He writes of what he has seen, with many interesting observations on bird conduct and behaviour, he gives us facts concerning distribution, breeding places, etc., and tells about his visits to many of the great seabird haunts in England, Scotland, Sweden, Finland and so on, his delight being the spectacular species that may be found on cliff, shore and island.

The author's style is concise but pleasant, and he has the gift of vivid description. We must not forget this is a translation from the French in which it was written and first published; however, it does not read as if it has lost anything in the process.

The latter part of the book is devoted to "Descriptive Summaries" in which the facts concerning each bird are set out concisely so that they may be seen at a glance.

Reverting to the pictures, which are all good, it is difficult to pick out one for special mention, but the photograph of an arctic tern on p. 189 is a remarkable study of a bird in flight, every detail, every feather, being depicted of the spread wings and spread tail.

A parent or other person wishing to give a boy or girl a present could not do better than choose this book, for it will surely fire youthful enthusiasm.

FRANCES PITT

CRYSTALLOGRAPHY CONDENSED

General Crystallography

A Brief Compendium. By W. F. de Jong, with the collaboration of J. Bouman. (Series of Books in Geology). Pp. ix+281. (San Francisco: W. H. Freeman and Company, 1959.) 38*s*.

ORIGINALLY published in Dutch in 1951, this work has now been enlarged and partially re-written for an English language edition. The primary object was to provide a review of the whole subject of crystallography; in the preface the author suggests that his book may also be used as a guide in private study, and by undergraduates as a summary of modern ideas on the subject. The book is in four parts, the first and longest (92 pages), on geometrical crystallography, being followed by sections on

structure theory and determination (43 pages), crystal chemistry (53 pages) and crystal physics (79 pages).

To review a whole science in a small space is a task calling for the most careful selection and presentation. The author has chosen to make his book reasonably encyclopædic, keeping down the length by a terse style of writing, by the use of numerous excellent diagrams, and by a multitude of references to more specialized works. The selection has, in fact, been a local, as against a regional, one. Considered as a compendium rather than a text-book and in view of its length, this is a successful book, remarkably complete and accurate. The brevity of the presentation has many drawbacks, of course; thus results are often stated without proper derivation, and the mathematical sections inevitably make dull reading. In the more descriptive chemical and physical sections the *hors d'œuvres* presentation certainly whets the appetite, but for the main course one is referred to other books. The section devoted to the explanation of crystal properties in terms of atoms is the most original and interesting one with a wide scope, including such topics as Frank–Read sources of dislocations, Raman radiation and transistors. Even the granitization theory gets a brief mention, one of the few reminders that this book is part of a geological series.

The proper balance for a work of this kind is a matter of opinion, but probably most English-speaking crystallographers will feel that the theoretical side here outweighs the experimental one, and that the geometrical section in particular is far too heavy for the rest.

A few detailed criticisms may be made. The author goes against well-established nomenclature in certain places. For example, he uses 'solid solution' for what would normally be called an interstitial structure, the usual solid solution becoming a 'substitutional structure'. Again, (*hkxl*) is used in place of (*hkil*) for four-axis Miller–Bravais indices with no real justification. Whatever the reasons for such changes, it is a mistake to introduce them in a reference book.

For the professional crystallographer this book will have some value for reference, particularly for the geometric part, as this is otherwise mostly treated in German texts. As a guide to private work it would be hard going without advice on selection. Parts of the book will be valuable to the undergraduate for revision or summary, while others are too advanced.

M. G. Bown

GEOLOGY IN THE UNITED STATES

Geological Survey Research 1960

Professional Paper 400, U.S. Geological Survey. Vol. A, Synopsis of Geologic Results. Pp. xii+136. 1 dollar. Vol. B, Short Papers in the Geological Sciences. Pp. viii+515. 4.25 dollars. (Washington, D.C.: Government Printing Office, 1960.)

FOR many years the United States Geological Survey has had a greater output of research in the Earth sciences than any other organization in the world. The immense mass of its publications, embracing the work of close on a thousand geologists, coupled with the inevitable delays in printing long detailed reports, has, however, resulted in some of its work not being known as widely or as speedily as it deserves to be; and to provide a more prompt and succinct release of new information the Survey has now issued this summary of progress which, if found useful, will henceforth be published annually. The publication is in two quarto volumes. The first presents a narrative synopsis of the technical activities of the Geologic Division during the fiscal year ended June 30, 1960. The second comprises 232 papers, most of them less than 1,000 words in length, which are either announcements of new discoveries on problems of limited scope or reviews of the results of extensive investigations that have been in progress for some time.

In his preface Dr. T. B. Nolan, director of the U.S. Geological Survey, states that "This report is frankly an experiment". The experiment is a highly successful one, for all but the most parochial workers in the geological sciences will find in these columns contributions within their fields of interest. The short papers are concerned with economic geology (42 contributions), exploration and mapping techniques (20), geology applied to engineering and public health (11), regional geology mainly of the United States (101), palæontology (6), geophysics (14), mineralogy, chemistry, and petrology (20), and analytical or petrographic methods (18). Notwithstanding the remarkable rapidity in publication a high editorial standard is maintained throughout; and Dr. V. E. McKelvey, who planned and directed the preparation of the volumes, is to be congratulated on the overall lucidity of presentation. Undoubtedly this review of progress will henceforth be one of the high-lights of the geological year; and by setting an example it should do much to improve and speed up the annual reports of other national surveys.

C. F. Davidson

COAL SCIENCE: AN INDIAN VENTURE

Proceedings of the Symposium on the Nature of Coal

Held at the Central Fuel Research Institute, Jealgora, 7th–9th February, 1959. (Organized by the Central Fuel Research Institute in collaboration with the Institute of Fuel, Indian Section.) Pp. viii+321. (Jealgora: The Central Fuel Research Institute, undated.) n.p.

A REPORT of the symposium on "The Nature of Coal", held at the Central Fuel Research Institute, Jealgora, has already appeared in these pages (*Nature*, **183**, 1438; 1959); now the proceedings have become available.

The volume is well produced and I have come across few typographical errors; all papers are written in English. As often with such collections, more than a year has elapsed between conference and publication of the proceedings; as a result, some sections, especially that on the rapidly developing subject of coal chemistry, no longer represent the latest views on all aspects considered.

The conference was divided into six sessions as follows: origin and systematics of coal; petrographic and X-ray studies on coal; ultra-fine structure of coal; coal constitution, physical methods; coal constitution, chemical methods; physico-chemical properties of coal.

The attendance from Western European countries, North America and Australia was unfortunately small, and most of the papers from these areas, for example those by H. Barking and C. Eymann, N. Berkowitz, J. D. Brooks, I. G. C. Dryden, R. A. Durie and S. Sternhell, C. Kröger and Dobmaier, and M. Samec, could not therefore receive adequate discussion. A paper from the Canadian Bureau of Mines and Technical Surveys was presented by one of the authors, B. N. Nandi. To judge from the printed discussion, similar difficulties limited the consideration given to papers from the U.S.S.R. and eastern Europe, of which there were nine distributed over the first five sessions, mainly in the first, and of those from Japan (three). Half the forty papers presented originated outside India.

The selection of papers with especial interest is invidious and must be to some extent personal to the reviewer. However, I would mention three: "Carbon-bound Methyl Groups and the Mechanism of Coalification", by Kaiser, Rao and Lahiri in particular; "Pyrolysis and its Relation to the Structural Parameters of Coal", by Mazumdar, Chakrabartty and Lahiri; and "Relation between Chemical Constitution and Pyrolysis of Coal", by Biswas, Sarkar, Kini, Das Gupta and Lahiri. Less personal perhaps is surprise at the widespread tendency to record chemical analyses to two places of decimals when only one is justified by the available analytical techniques. It is disappointing to find only eight pages of discussion on three hundred pages of papers presented. Despite the title of the symposium, the technology of the applications of coal is not unrepresented. The whole volume is of considerable interest both for reading and reference.

I. G. C. Dryden

PESTS AND THEIR CONTROL

Horticultural Pests

Detection and Control. By G. Fox Wilson. New edition, revised by Dr. P. Becker. (Agricultural and Horticultural Series.) Pp. xix+240+4 plates. (London: Crosby Lockwood and Son, Ltd., 1960.) 25*s*. net.

Chemical and Natural Control of Pests

By E. R. de Ong. Pp. viii+244. (New York: Reinhold Publishing Corporation; London: Chapman and Hall, Ltd., 1960.) 60*s*. net.

Ten Little Housemates

By Karl von Frisch. Pp. 146. (London and New York: Pergamon Press, 1960.) 17*s*. 6*d*. net.

THE late George Fox Wilson was a garden lover and economic entomologist with profound experience of the pest problems that beset the gardener, and the rare gift of being able to write clearly, accurately and interestingly for the layman. His book, "The Detection and Control of Garden Pests", was therefore well received by all. In agreeing to revise it, Dr. Paul Becker not only had to stand the test of comparison with his predecessor, but also to bring the book up to date with recent rapid developments. He has been outstandingly successful.

The book has a new title, but retains its original form and content. Additions have had to be made: many new chemicals have come into use, and perhaps as a result of their misuse we have been forcibly reminded of the existence and importance of the natural enemies of insects. Thus Dr. Becker has rewritten the section on biological control and very sensibly continued to stress the importance of cultural and other practices that reduce the need to use insecticides.

There are more illustrations and the excellent quality of the paper used has made the most of them. The colour photographs, mostly provided by the Shell Photographic Unit, are exquisite.

Except for the few who correctly foresaw the problems that might arise, the dawn of the synthetic insecticide era seemed to be heralded by many economic entomologists as the beginning of the end of our insect competitors. Within a few years, however, it appeared likely that insecticides were creating as many problems as they solved, and we became only too familiar with such things as pest resurgence and resistance to insecticides. Such things were not really new, but in the excitement of new chemical discoveries they were often forgotten. Perhaps the most important by-product of this era was the stimulus it gave to ecological and other studies of pests, and to thoughts about the rational use of insecticides. That is, to try to put it briefly, how these undoubtedly valuable chemicals can best be used in combination with cultural and other practices to minimize pest damage without interfering with the benefits that may be derived from the activity of beneficial insects, or of inducing other undesirable side-effects. This approach is clearly reflected in Dr. Becker's book, and a great deal of fascinating work has been done along these lines in North America and in Europe.

If the title of Dr. E. R. de Ong's book leads the reader to expect a balanced account of recent work on rational pest control he will be disappointed. This book deals mainly with the control of pests (insects, diseases and weeds) of crops and livestock, of the household and of storage in the United States. There is little doubt that the recommended measures are sound, and that the quite well-arranged information will be of use to the American extension worker, but of much less value in the United Kingdom.

In "Ten Little Housemates" Prof. Karl von Frisch has written a series of essays that give the impression that he enjoyed writing them. If the purist is prepared to tolerate a work that makes no pretence of being a scientific treatise, no doubt he will be able to enjoy them too. It is written for the layman who takes an intelligent interest in what he sees around him, and for him this little book can be recommended.

F. H. Jacob

GROWING POINTS IN PLANTS

Gründzuge der Histogenese Höherer Pflanzen

Von Prof. Dr. Hermann von Guttenberg. 1: Die Angiospermen. (Handbuch der Pflanzenanatomie, Band VIII, Teil 3. Zweite, völlig neubearbeitete Auflage herausgegeben von Prof. Dr. W. Zimmermann und Prof. P. G. Ozenda.) Pp. viii+315. (Berlin-Nikolassee: Gebrüder Borntraeger, 1960.) 110 D.M.

THIS book is about the patterns of cells in the apical meristems of roots and shoots although its title may lead us to expect something else. It falls into three main parts, dealing with roots, shoots

and leaves, and there is a short section on a few anatomical aspects of differentiation. Prof. Guttenberg is well known for his work on root apices, and, for some people, the most useful part of the book will be the summary of his views on these, but the other two main sections are useful, painstaking accounts of developmental anatomy of the comparative kind. The book is profusely illustrated with modern line drawings and a few badly reproduced photographs.

It is marred by an almost total exclusion of any work which is not a simple consideration of cell patterns and by a dogmatic assertion of the author's views on these. He has not appreciated that merely putting some arrows on a diagram is not good evidence that cells behave in the way he says they do. Prof. Guttenberg does not like my interpretation of root meristems, and, to attack this, he ascribes to me views which I do not hold and does not try to explain the facts which, in spite of his protestations, are inconsistent with his own interpretation. Similarly, he has missed the significance of Thielke's important work on chimeras and of some American work on shoot apices. There are also some extraordinary gaps in some of the work presented and quite a lot of minor errors. There are, however, adequate indexes.

The book is likely to find a place in reference libraries partly because it is one of a reputable series of volumes on plant anatomy and partly because of its value as a comparative account of apical structure. Its price is likely to keep it out of personal libraries in Great Britain. Its old-fashioned approach is not likely to inspire a young biologist to study apices, and this is a pity to-day when there are so many opportunities for useful work on the behaviour of meristems. F. A. L. Clowes

FLORA OF TROPICAL EAST AFRICA

Loganiaceae

By E. A. Bruce and J. Lewis. Edited by C. E. Hubbard and E. Milne-Redhead. Pp. 47. (London: Crown Agents for Overseas Governments and Administrations, 1960.) 4*s*.

THE "Flora of Tropical East Africa", of which the parts dealing with the families Loganiaceae by Mr. J. Lewis and the late Miss E. A. Bruce, Rosaceae by the late Mr. R. A. Graham, and Alismataceae and Butomaceae by Miss S. Carter have been published this year, covers the territories of Kenya, Uganda, Tanganyika and Zanzibar, an area of about 707,000 square miles in all. The Flora, which is being prepared under the editorship of Dr. C. E. Hubbard and Mr. E. Milne-Redhead, is being published family by family in separate parts with individual paging and pricing. So far 30 parts, including an introductory part and a glossary, have been published. Although the work is centred at Kew, much of it has been carried out by staff appointed by the Colonial Office for work on this Flora.

The Flora itself contains keys both to the genera and species, and also where necessary to sub-species and varieties; in addition, there are descriptions which are not intended to be exhaustive, but sufficient to indicate the main features of importance in identification of the entities keyed. References to the most important literature, particularly that of concern in East Africa, are also given, and representative specimens from the territories in which each species occurs are cited, usually only a limited selection of the total number seen. There are, in addition, brief notes on the habitat of each species in East Africa and its distribution both in East Africa and elsewhere. As a great deal of new taxonomic research has been involved, many specific names hitherto accounted distinct are here reduced to synonymy for the first time, and the lists of synonyms (primarily those relating to East Africa) will be found to contain much otherwise unpublished information. Each part of the Flora is illustrated with black-and-white figures so as to represent the habit of at least the majority of the genera described. There are also comparative figures bringing together for comparison diagnostically important organs of a series of species. As the total number of species in the area is probably in the neighbourhood of eleven thousand, it will be realized that the labour involved in working out this Flora will be both massive and lengthy.

The area covered by this Flora is, at least for Africa, unusually varied both in climate and altitude, including different sorts of vegetation ranging from the rain-forest, found here and there in Uganda and north-western Tanganyika and still more rarely and locally elsewhere, to the semi-desert scrub of the northern parts of Kenya, and the wonderful afro-alpine vegetation of the high mountains of East Africa. Perhaps the majority of the area is covered by the different aspects of the grassland, wooded grassland and woodland that clothe such huge tracts of tropical Africa. On this variety of vegetation, which often reflects differences in climate, there is superimposed a variety of different floristic elements: the rain-forest flora the species of which are mostly typical of the Congo and West Africa, the rich and remarkable flora, often containing numerous endemics, in the patches of forest scattered near the coast, the Somalian flora in northern Kenya, which though perhaps not rich in species, contains, however, many found nowhere else but in north-eastern Africa, the remarkable mountain flora so well represented in East Africa, and the generalized woodland and grassland flora so typical of much of east and south-tropical Africa may be taken as examples.

One of the interesting results coming from the taxonomic research involved in this Flora is that many species are being shown to occupy unexpectedly wide areas in tropical Africa, and to display ranges of variation which may frequently be far-reaching and complicated. What is now shown to be a single, perhaps polymorphic species may in the past have been known under several different names, each corresponding to a different part of its total geographical range. This has often given rise to false and misleading ideas about endemism and the size of genera. The patterns of variation take many different forms, and may have originated in various ways, perhaps through the intrinsic variability of certain species, or perhaps through the swamping of originally distinct and separate species which have come into contact with one another for some cause, giving rise to numerous hybrids destined to outnumber their parent species. The presence of such problems is obvious enough, but the evidence is usually insufficient for more than guessing at their cause.

J. P. M. Brenan

Nuclear Radiation Measurement

By J. Sharpe. (Nuclear Engineering Monographs.) Pp. vii+71. (London: Temple Press, Ltd., 1960.) 12*s.* 6*d.* net.

THIS book is one of a series of short monographs on nuclear engineering subjects intended for university and technical college students, research assistants and qualified technicians. With only sixty-six pages of text the author has concentrated on outlining the physical principles by which the various detectors operate, and there is little practical information on their design and construction. Each of the four main chapters has a useful list of references, and there is a short index.

The first main chapter, which occupies one-third of the book, discusses the way by which charged and uncharged particles and electro-magnetic radiations interact with matter. The various processes of energy absorption are mentioned, and some relevant data are presented in tabular form. Emphasis is placed on the scintillation phosphor as a detector, and the properties of different types of phosphor are examined in some detail.

The next chapter describes the operating mechanism of different types of detector, including a mention of such specialized devices as cloud- and bubble-chambers and photographic emulsions. Perhaps inevitably, the treatment of the more common gas-ionization detectors (ion chambers, proportional counters and Geiger–Müller counters) is somewhat incomplete; there is a short section on multiplier photo-cells. There follows a small section listing the ancillary equipment necessary for the working of the detectors, such as power supplies, amplifiers, scalers, etc., and then the last main chapter considers the types of detector best suited for a number of specific applications. Mention is made of α-particle assay using a zinc sulphide phosphor or a flow proportional-counter, and the absolute and relative measurement of β-particles.

The measurement of the energy of gamma radiation by scintillation counters is described, and the use of X-ray proportional counters for low-energy X-radiation is mentioned. Detectors employing the boron-10 (n,α) reaction for thermal neutron measurement are described, and some techniques for the detection of fast neutrons are noted.

The book is clearly written and produced, and fulfils its aim of introducing the very wide subject to technical students and research assistants.

W. R. LOOSEMORE

Anorganische Chemie

Band 1. Von István Náray-Szabo. Pp. 664. (Budapest: Verlag der Ungarischen Akademie der Wissenschaften (Akadémiai Kiado), 1959.) n.p.

PROF. SZABO'S book is planned to extend to three volumes. It begins with a good, concise account of atomic structure, radioactivity, and the theory of valency. The author believes that a book on inorganic chemistry of this size should confine itself to its subject and not branch off into disquisitions on physical chemistry or organic chemistry. The elements are dealt with systematically in the order of the Periodic Table, the compounds being in a systematic order. There are tables of physical properties, an arrangement which saves space and is useful for reference. In a few places the treatment requires revision, for example, in the description of non-existent substances such as FO. The translation from Hungarian into German makes the book accessible to a large class of readers, and it deserves to be successful.

J. R. PARTINGTON

Chemical Instrumentation

A Systematic Approach to Instrumental Analysis. By Dr. Howard A. Strobel. (Addison-Wesley Series in Chemistry.) Pp. xviii+654. (Reading, Mass. and London: Addison-Wesley Publishing Company, 1960.) 74*s.*

THIS is a volume of 653 pages, printed legibly on excellent paper, and it presents, in small compass, an extensive store-house of information on the theoretical and practical aspects of most known fields of instrumental analysis. Its range of topics is so incredibly wide that, from the practical laboratory point of view, some useful detail has, of necessity, been omitted. Nevertheless, as a book for the undergraduate or honours degree student it has much to commend it.

The theoretical approaches are excellently described; the compilation of methods has been admirably done, and the many diagrammatic illustrations are clear and singularly helpful.

Most fields of spectroscopy are succinctly described: radioactive techniques, polarography, gas chromatography, polarimetry, electrometric methods, etc., all find their place in the various chapters. Scientific fundamentals are never ignored; there are two chapters on basic electronics, including transistor circuitry, and the information contained therein materially assists the student in the real understanding of the instrumental methods covered in the text. Another interesting chapter deals with "Errors of Measurement". The book concludes with a suggested series of detailed laboratory experiments which parallel in diversity the topics covered by the author.

It is not often that the student can purchase so much scientific information at such a modest price.

D. T. LEWIS

The Bridal Bed

By Joseph Braddock. Pp. 189+8 plates. (London: Robert Hale, Ltd., 1960.) 18*s.* net.

THIS book is really an anthology, culled from a great variety of authors, some reliable and some otherwise, concerning marriage ceremonies in many parts of the world.

The book is divided into six parts. In the first the choice of the marriage partner is described, while in the second courtship as seen in both civilized and primitive communities is described in some detail. From these preliminaries spring naturally accounts of the wedding itself; and finally some consideration is given to what various writers have said about consummation and customs relating to the various human relationships within the marriage state.

For those who wish to gain a general idea of what has been said about marriage customs in both Western and Oriental countries this anthology would provide an excellent introduction, although it would have been much improved had more trouble been taken to verify references and avoid relying on authors whose inaccuracy must surely have been apparent. The book is well printed and arranged, although it is not easy to understand why the bibliography is not arranged in correct alphabetical order.

E. J. DINGWALL

LOW-TEMPERATURE FORMS OF ICE AS STUDIED BY X-RAY DIFFRACTION

By L. G. DOWELL and A. P. RINFRET

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IN recent years a number of investigators have studied the low-temperature, low-pressure structures of ice, using such techniques as thermal analysis (Pryde and Jones[1]), electron diffraction (König[2]), and X-ray diffraction (Barnes[3]). A comprehensive treatment of the literature is given by Blackman and Lisgarten[4]. General agreement exists that there are three possible phases of ice at low temperatures and pressures, namely, hexagonal or ice I, diamond cubic, and vitreous. There is, however, wide divergence of opinion as to the details of formation of these phases and to the conditions for their existence. We have determined the temperature-ranges in which the various phases exist and the rates of transformation from one phase to another. We have been able to produce vitreous ice only by condensing water vapour on a surface maintained below − 160° C. This vitreous ice undergoes a partial and irreversible change to cubic ice at temperatures above − 160° C., the rate of transformation being strongly dependent on temperature. The mixed cubic-vitreous phase undergoes an irreversible transformation to hexagonal ice I at temperatures above − 130° C., the rate also being dependent on temperature.

The basic X-ray equipment consisted of a water-cooled diffraction unit (Norelco Type 12045), a copper target X-ray tube, a scaler-ratemeter (Norelco Electronic Circuit Panel, Type 12096), a linear amplifier (Norelco Main Amplifier Chassis, Type 42234) and pulse-height analyser (Atomic Model 510). The diffractometer was built in our own shops but closely resembled the Norelco Type 42266. It was equipped with a flow-type proportional counter using an argon–methane (90 : 10) gas mixture.

A low-temperature vacuum chamber attachment was constructed as a replacement for the regular sample holder. It consisted of a hollow copper tank (Fig. 1) containing a depression for the sample plate and was attached to a phenolic block which was in turn attached to the sample shaft. An O-ring provided a vacuum seal between the vacuum chamber back-plate and the phenolic block. The back-plate carried Kovar seals for the thermocouple leads and also carried the refrigerant lines. The vacuum cover was sealed to the back-plate with an O-ring and contained slots covered with polystyrene sheets as the X-ray windows. The vacuum connexion was also in the cover. The copper–constantan thermocouple was cemented to the sample plate with silver paint and was located just outside the area irradiated by the X-ray beam. A vapour inlet tube was inserted into the vacuum cover, and its tip was located 1 in. above the centre of the sample plate. Finally, a 0·00035-in. nickel foil sheet was placed over the depression in the tank and served both as a thermal radiation shield and as an X-ray filter.

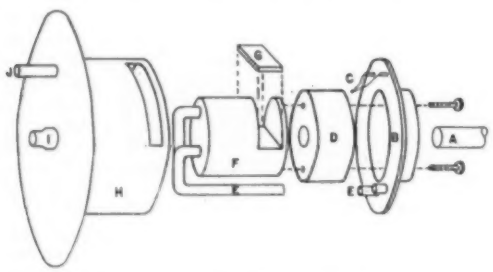

Fig. 1. Low-temperature cell for the X-ray diffractometer *A*, diffractometer sample shaft; *B*, vacuum chamber base plate; *C*, thermocouple; *D*, phenolic block; *E*, refrigerant lines; *F*, hollow cold cell; *G*, specimen plate; *H*, vacuum chamber cover; *I*, vacuum connexion; *J*, water vapour inlet

The vacuum system consisted of a rotary oil-sealed pump which maintained a vacuum estimated at about 5μ except during sample preparation. This pressure provided adequate thermal insulation yet did not cause noticeable sublimation of the sample except at temperatures above −30° C. The total heat leak was such that the rate of warming was about 3 deg. C. per min. at − 160° C. and less than 2 deg. C. per min. at − 90° C. when the refrigerant supply was discontinued.

Two refrigerant systems were used. For investigations near − 190° C., liquid nitrogen was forced through the refrigerant lines directly into the cold cell. The minimum temperature obtained was − 192° C. For most of this work, however, the various temperatures were maintained by means of a stream of nitrogen gas which first passed through a copper coil immersed in a Dewar flask of liquid nitrogen before moving into the cold cell. By controlling the rate of flow of the gaseous nitrogen, any temperature down to − 175° C. could be maintained as long as needed.

Ice Structures

Hexagonal phase. Much has been published concerning the structure of ordinary hexagonal ice (usually called ice I), and the reader is referred to Owston[5] for an excellent review of this literature. We will discuss the simple Barnes model of ice which shows only the oxygen arrangement in the lattice, since the hydrogen atoms contribute so little to the X-ray diffraction pattern.

One may conveniently consider the hexagonal model as being built up of layers of oxygen atoms, each layer consisting of a network of open puckered hexagonal rings. Each additional layer is a mirror image of the preceding layer, so that the stacking is of the $A \cdot B \cdot A \cdot B \cdot \cdots$ sequence. It can be shown that this leads to the tetrahedral arrangement of atoms of oxygen known to exist in ice I. The c_0 parameter is twice the layer spacing and was found to be 7·338 ± 0·004 Å. at − 175° C., while the a_0 parameter was 4·498 ± 0·002 Å. There are four

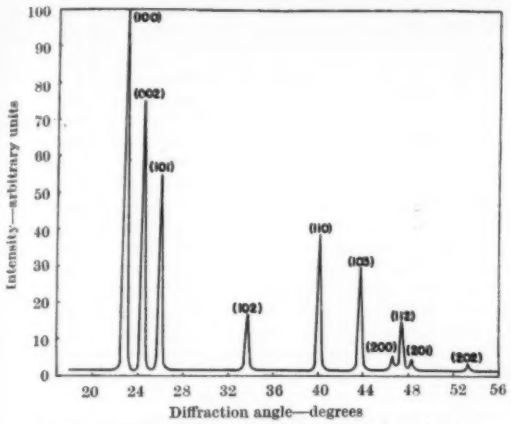

Fig. 2. X-ray diffraction pattern of hexagonal ice (ice I)

molecules per unit cell, leading to a computed density of 0·924 gm./c.c. A typical X-ray diffraction pattern of this phase is shown in Fig. 2, and Table 1 shows the d values and relative intensities of all lines observed up to an angle of 56° (2θ). The lines are quite sharp, indicating no line broadening due to small particle size. The variation of intensity ratios from experiment to experiment indicates either the presence of large particles or of preferred orientation of somewhat smaller particles. The relative intensities listed in Table 1 are the averages of several typical patterns of this phase.

Table 1. X-RAY DIFFRACTION DATA FOR HEXAGONAL ICE AT − 175°C.

hkl	2θ (deg.)	*d* (Å.)	I/I_0
100	22·82	3·897	100
002	24·26	3·669	75
101	25·89	3·441	53
102	33·55	2·671	17
110	40·09	2·249	39
103	43·70	2·072	30
200	46·62	1·948	4
112	47·41	1·918	18
201	48·34	1·883	3
202	53·24	1·721	2

Diamond cubic phase. König[2] was probably the first to recognize and deduce the structure of cubic ice. Shallcross and Carpenter[6] confirmed the identity of this structure, describing the conditions for its formation as well as its properties. The oxygen atoms are located in a diamond cubic lattice, but the structure may also be considered as being built up in layers of oxygen, each layer identical with that of the hexagonal phase. However, each successive layer is shifted one-half the diameter of the hexagonal ring, leading to the $A \cdot B \cdot C \cdot A \cdot B \cdot C \cdot \cdots$ stacking sequence typical of the diamond cubic system.

We have found the cubic parameter to be $a_0 = 6{\cdot}358 \pm 0{\cdot}004$ Å. at − 185° C., leading to a computed density of 0·923 gm./c.c. This density is identical with that of the hexagonal phase within experimental error. The similarity of the structures of the two phases is more easily seen by deducing the pseudohexagonal cell of the cubic phase and comparing it with the hexagonal phase (Table 2). It will be noticed that the a_0 parameters are the same, but the c_0 parameter of the pseudohexagonal cell is 50 per cent greater, leading to the same layer spacing for both phases.

Table 2. CRYSTAL PARAMETERS FOR HEXAGONAL AND CUBIC ICE

	Hexagonal	Cubic	
		Diamond cubic cell	Pseudohexagonal cell
a_0	4·498 Å.	6·358 Å.	4·495 Å.
c_0	7·338 Å.	—	11·012 Å.
c_0/a_0	1·631	—	2·450
Layer spacing	3·669 Å.	—	3·671 Å.
Density	0·924 gm./cm.³	0·923 gm./cm.³	0·923 gm./cm.³

A typical X-ray diffraction pattern of the cubic phase is shown in Fig. 3, and Table 3 lists the d values and intensities of all lines appearing up to 56° (2θ). The small peak appearing to the left of the (111) peak is partly due to a trace of hexagonal ice and partly to the maximum of the residual vitreous ice. The intensity ratios are quite reproducible and the lines show considerable broadening. Application of the Scherer law gives an average particle size of about 400 Å. This same result is obtained regardless of the rate or temperature of transformation from the vitreous phase.

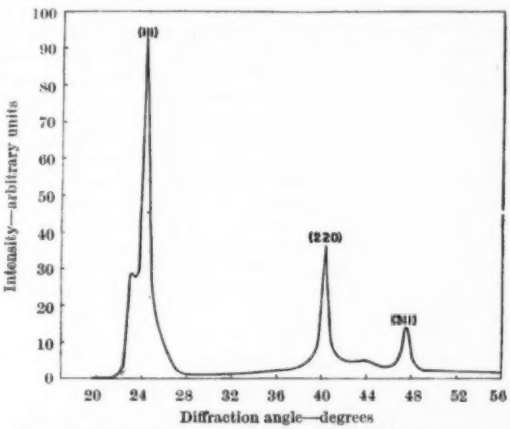

Fig. 3. X-ray diffraction pattern of diamond-cubic ice in the presence of vitreous ice

It should be pointed out that we have never observed the cubic phase in a pure state, but always with what has been considered as a modification of the original vitreous phase. This aspect of the problem is examined in the following section.

Vitreous phase. Fig. 4 shows a typical X-ray diffraction pattern of vitreous ice. It is characterized by two intensity maxima, one at 24° (2θ) corresponding to a Bragg spacing of 3·71 Å. and having a half-width of 5·0°, and the second at 42° (2θ) corresponding to 2·15 Å. Dorsey[7] has compiled a list of X-ray diffraction patterns of liquid water reported by a number of investigators. These data indicate periodicities in the structure having values of 3·20 Å, 2·11 Å. and 1·35 Å. Although our observed value of 2·15 Å. and the reported value of 2·11 Å. are in

Table 3. X-RAY DIFFRACTION DATA FOR CUBIC ICE AT −185° C.

hkl	2θ (deg.)	*d* (Å.)	I/I_0
111	24·25	3·670	100
220	40·11	2·248	40
311	47·43	1·917	15

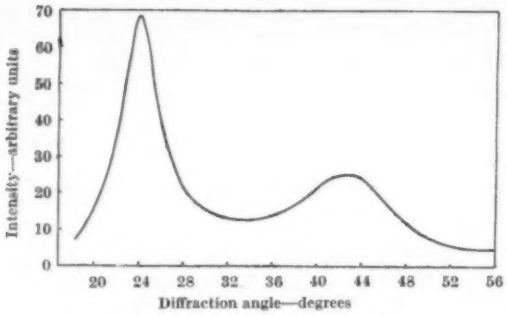

Fig. 4. X-ray diffraction pattern of vitreous ice

agreement, the 3·71 Å. observed value is unique. It is therefore evident that the structure of vitreous ice is different from that of water at ordinary temperatures. Further discussion of the structure of this phase is found in the final section of this article.

The portion of the original vitreous ice which remains after the cubic transformation is complete has also been altered. If the shape of the vitreous diffraction curve near 24° (2θ) is observed during the transformation to the cubic phase, it is found that the centre of the curve remains virtually unchanged, whereas the intensity gradually diminishes on the high-angle side and more abruptly on the low-angle side. At the same time, the relatively sharp cubic (111) line appears on the crest of the vitreous maximum. When an estimated 30 per cent of the vitreous ice has been transformed to the cubic phase, the conversion ceases regardless of the time or temperature involved. In addition, this same pattern is observed when the original condensation is carried out at temperatures sufficiently high to form the cubic phase directly.

Production of Vitreous Ice

Several attempts were made to produce vitreous ice from liquid water, but none was successful. They included spraying water on a substrate held at − 190° C., and plunging films of water on metal foils into liquid nitrogen. Only normal hexagonal ice resulted, as might be expected from Luyet's[8] prediction that a cooling-rate of 20,000 deg. C. per sec. would be required to vitrify liquid water.

In another method, atmospheric moisture was collected on the refrigerated sample plate. Small amounts of vitreous ice were obtained; but, as the deposit formed, the surface temperature rose due to the insulating effect of the ice layer and the crystalline forms of ice appeared, as did solid carbon dioxide.

The condensation of pure water vapour is the most reliable and practical method of producing vitreous ice. Although the direct condensation of vapour from a liquid or solid source has been used by most investigators, we have found that greater control can be exercised by using a stream of wet gas. We caused a small stream of nitrogen gas to pass through a spiral gas-washing bottle containing distilled water and then through a needle valve into the vapour inlet tube built into the vacuum cover of the low-temperature cell. The wet gas impinged on the centre of the cold sample plate, where the water vapour was condensed and the nitrogen was continuously pumped off. The thickness of the ice film was computed by measuring the decrease in intensity of the diffraction pattern of the silver sample plate. At a rate of flow of 75 c.c. per min. of wet nitrogen, a layer of ice 60μ thick was deposited in 30 min.

No attempt was made to determine accurately the conditions for the formation of the various phases from the vapour state. However, we found that vitreous ice could be formed at any temperature below − 160° C., while various amounts of cubic ice appeared if condensation occurred at higher temperatures. Above − 120° C. a very highly oriented film of hexagonal ice formed. We also found 60μ to be the maximum thickness of vitreous ice obtainable. This was also probably due to the high surface temperature acquired as the deposit thickened.

Most of the work reported here was carried out using a polycrystalline silver plate for the substrate. To test the substrate effect several trial experiments were made using a glass plate, but in no part of the entire procedure was any difference noted. It is concluded that our results are free of any substrate effects.

Phase Transformations

Vitreous to cubic. This irreversible phase change was observed to be strongly dependent on temperature and took place in the temperature range − 130° to − 160° C. At higher temperatures, the conversion is very rapid and no measurements of rate were made. The rate of transformation was measured by setting the X-ray diffractometer at the correct angle for the cubic (111) line and observing the increase in intensity as a function of time at various temperatures. The transformation appears to proceed linearly with time, until it nears completion, when it suddenly slows. The conversion times were obtained by extrapolating the main part of the time – intensity curves to the maximum intensities when equilibrium was reached. It should be noted that this occurs when about 30 per cent of the original ice has been converted, as estimated from the area under the vitreous curve and the peak heights of the crystalline phases. For the lower temperatures the scaler and timer were used periodically to determine the increase in counting-rate with increasing time. The temperature was then raised to − 120° C. for a short time to ensure the maximum conversion, the counting rate was measured, and the transformation time was computed. Equation 1 relates conversion time and sample temperature in the range of − 135° C. to − 160° C.:

$$t = 2{\cdot}04 \times 10^{23} \times \exp(-0{\cdot}465T) \qquad (1)$$

where t is the time in minutes for the conversion and T is the absolute temperature in degrees Kelvin. A graph of observed values is shown in Fig. 5. The transformation occurs rapidly above − 135° C., and if the temperature of a specimen of vitreous ice is raised at a rate of 5 deg. per min., the conversion appears to take place suddenly near − 130° C.

A single determination of rate was carried out at − 160° C., but since the 12-hr. observation period was a small fraction of the computed transformation time of 205 days, we do not consider that observations at this temperature are reliable. One approach to this problem is to store a specimen for weeks at − 160° C. and lower, and to examine it periodically by X-ray diffraction methods to determine whether our equation is valid for lower temperatures.

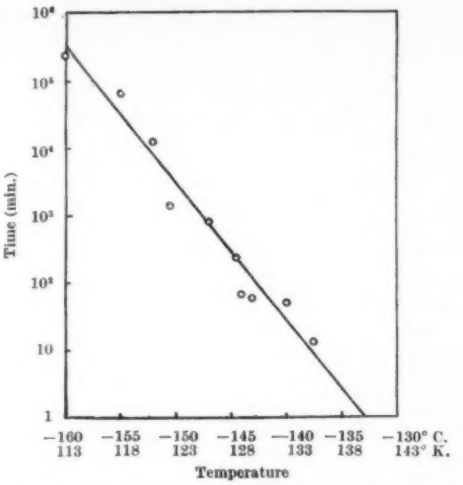

Fig. 5. The time necessary for the conversion of vitreous to cubic ice

Cubic to hexagonal. A further increase in temperature above − 130° C. allows transformation to the hexagonal phase. Although it is certain that the residual vitreous ice becomes converted to hexagonal ice, the evidence that the cubic phase is also converted is indirect. All three diamond cubic lines exactly coincide with hexagonal lines, and the large particle size and possible orientation effects of the hexagonal phase make the analysis of intensity ratios a poor guide to the presence of the cubic phase. However, the sharpness of the hexagonal lines and the lack of broadness near the base of the lines show that the cubic ice, if present, has appreciably increased in particle size. We conclude that probably both the vitreous and the cubic forms are converted to the hexagonal phase, and we will call it the cubic–hexagonal transformation.

The rate of this transformation is also dependent on temperature, although much less sensitive in this respect than is the vitreous–cubic change. The rate was measured with the same procedure described earlier, except that the diffractometer was set at the angle for the (100) line of hexagonal ice. Equation 2 relates time of conversion and sample temperature in the range of − 65° to − 120° C.:

$$t = 2{\cdot}58 \times 10^{12} \times \exp(-0{\cdot}126T) \qquad (2)$$

with t in minutes and T in degrees Kelvin. The observed values are shown in Fig. 6. This transformation occurs rapidly above − 70° C., and if the temperature of a specimen of vitreous and cubic ice is raised at a rate of 5 deg. C. per min. the conversion takes place rather quickly near − 60° C.

A single determination of rate was carried out at − 120° C., but here also the time of observation was too short for us to obtain reliable results.

Discussion and Conclusion

The results presented require consideration of alternatives with respect to the actual structure of the vitreous phase. Of two possible interpretations of the observed diffraction pattern, one is that the 'vitreous' ice obtained by our procedure is composed of small particles of crystalline ice, so small that the diffraction maxima are greatly broadened. Supporting this view is the fact that the 3·71 Å. spacing is very close to the 3·67 Å. value of the (111) line of cubic ice. Further, the 2·15 Å. value is between those of the (220) and (311) lines of cubic ice, and could be due to the overlapping of these two lines. However, application of the Scherer formula to the 3·71 Å. peak gives a particle size of about 20 Å., or about 3 unit cells in length. Since ordering is even less in the other directions and since the very essence of the crystalline state is the regular repetition of the unit cell, we consider it without significance to refer to this as a microcrystalline phase.

The second interpretation of this type of pattern is that 'vitreous' ice really has a typical vitreous or random network type of structure. In agreement with this theory are the following facts:

(1) No sharp conversion temperature from the vitreous phase was observed. This is consistent with the slow development of a crystalline lattice from a disordered array of molecules as the temperature is raised.

(2) Below − 160° C., substrate temperature and the rate of deposition of vapour produced no change in the X-ray diffraction pattern. Since crystal size is directly related to temperature, changes in the sharpness of the maxima could reasonably be expected between −190° and −160° C. if a microcrystalline structure were characteristic of vitreous ice.

(3) During the vitreous – cubic transformation (− 160° to − 130° C.) the intensity of the cubic peaks changed but their half-widths remained unaltered. This supports the interpretation of phase change (to a structure of constant particle diameter) rather than crystal growth.

(4) An exotherm near − 130° C. was reported by Pryde and Jones[1]. This observation would be expected if associated with a change in order, but would not be expected if associated with the growth of larger crystals at the expense of their smaller neighbours.

The foregoing observations predict that if a sample of vitreous ice is rapidly warmed, for example, 5 deg. C. per min., the vitreous–cubic transformation should

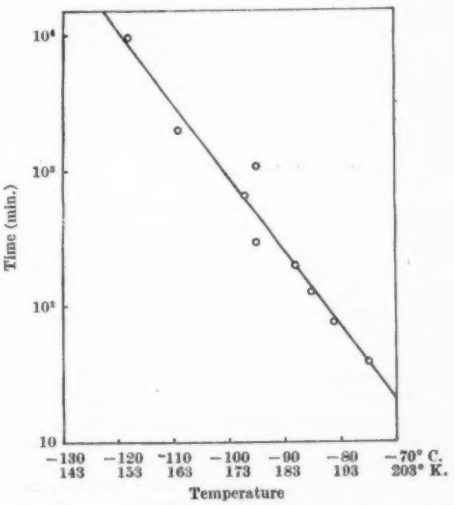

Fig. 6. The time necessary for the conversion of cubic to hexagonal ice

appear suddenly at about − 130° C. In a number of such experiments we have observed a sudden increase in the intensity of the cubic (111) line in the range −126° to −130° C., depending on the rate of increase of temperature. There can be little doubt that the exotherm reported by Pryde and Jones is due to this particular transformation and not to the vitreous–hexagonal one they have assumed. That we have observed only a partial transformation to the cubic structure is in agreement with their observation of a smaller amount of heat evolution during the phase transition than was expected on theoretical grounds.

If the fraction of the original vitreous ice that did not become converted to cubic ice is in fact vitreous, a second exotherm should be observed when it is converted to hexagonal ice. It is assumed that because of the structural similarity between the two crystalline phases very little heat will be evolved if or when the portion having the cubic structure is converted to the hexagonal. On the basis of the results presented for the cubic–hexagonal transformation, if the temperature is raised at about 5 deg. C. per min. through the − 90° to − 50° C. range, the second exotherm should appear at about − 60° C. Pryde and Jones, however, state that no thermal change (other than that at − 129° C.) occurred until the temperature reached 0° C. They did not include in their report the rate of warming of the specimen; but if, through the range −90° to −50° C., it was not greater than that near −130° C., it is likely that the exotherm would not have been observed. This is due to the relatively slow rate of crystallization to the hexagonal phase, a change which is far less sensitive to temperature than the vitreous–cubic transition. Slow crystallization would occur over a wide temperature-range making it difficult to observe an exotherm. To define more clearly the structure of the vitreous state, precise thermal measurements should be carried out on samples shown by X-ray diffraction techniques to be in that state..

We conclude that in vitreous ice domains exist in which there is a tendency toward the same layer structure present in the crystalline phases, although with random distribution of molecules within the layers. Those domains having very nearly the same layer spacing as the crystalline phases are apparently quite stable, but those having greater disorder are unstable. At temperatures above − 160° C., crystallization to the cubic phase begins spontaneously at a few sites in the unstable zones, and the crystallites at each site quickly grow to about 400 Å., where further growth is hindered by the more stable zones. Further crystallization takes place at new sites in the unstable domains, the process continuing until all such areas have crystallized. This state is now stable at all temperatures below − 130° C.

Above − 130° C. both the cubic and the remaining vitreous ice begin to show conversion to the stable hexagonal phase, the individual crystallites growing to moderate size, probably near 10μ. In view of the abundant evidence showing that ice crystals can grow in size at temperatures many degrees below the freezing point, it is reasonable to suppose that the ultimate size of the crystallites will depend on the highest temperature to which the sample is exposed for a given time.

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[2] König, H., *Z. Kristallog.*, **105**, 279 (1944).
[3] Barnes, W. H., *Proc. Roy. Soc.*, A, **125**, 670 (1929).
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[5] Owston, P. J., "Advances in Physics", **7**, 171 (1958).
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[7] Dorsey, N. E., ed., "Properties of Ordinary Water Substance", 320 (Reinhold Pub. Corp., New York, 1940).
[8] Luyet, B. J., "Cytology of the Blood and Blood Forming Organs", Bessis, M., 216 (Grune and Stratton, New York, 1956).

THE COLE LIBRARY OF ZOOLOGY AND EARLY MEDICINE, UNIVERSITY OF READING

IT has often been stated that the scientist is lacking in literary and artistic merit, yet this is far from the truth. Its complete falsity is demonstrated by the example of the renowned biologist, artist and historian of the evolution of biological knowledge and thought, the late Prof. F. J. Cole, who died in 1959 at the age of eighty-six. Whatever he did he made as perfect as possible, and his achievements in research, in the Department of Zoology which he founded at the University of Reading, the Museum which he built up, and the Library which he collected in his home, all testify to his greatness as a man of learning. When he retired from the professorship of zoology in 1939, the Council of the University resolved that the museum should be known as the Cole Museum (*Nature*, **177**, 555; 1956). This article deals with his library, henceforth to be known as the Cole Library.

Prof. Cole was a confirmed bibliophile. He collected books from his school-days onwards, throughout his long life. At first he bought the great monographs of biology, then turned more and more to the earlier works, so that at the end of his life he had built up a library of about eight thousand volumes, illustrating the history of early medicine and zoology from the fifteenth century to the present day. In 1932 he had a house built on the Chilterns, literally around the bookcases, and after his retirement from the professorship of zoology in the University of Reading in 1939, he compiled author, title and subject catalogues, before writing his major historical work, "A History of Comparative Anatomy from Aristotle to the Eighteenth Century" (ref. 1).

This Library, the most exhaustive one of its kind in private hands in the country, has recently been acquired by the University of Reading. For the present the books will remain in the late Prof. Cole's house, where they will be available to scholars as heretofore; but when the new University Library building, to be erected in Whiteknights Park, is completed, they will be transferred to a room set apart for the books and the catalogues, as the Cole Library, a fitting complement to the Department of Zoology and the Cole Museum at the University of Reading.

The two earliest books in the library are incunabula. Pliny's "Natural History" ("Caii Plynii Secundi Naturalis Historiae Libri Tricesimiseptimi") was printed in Venice in 1472. Although rebound in

vellum, it is in perfect condition, and has thirty-five coloured illuminated capitals and one full-page illumination. Aristotle's "De Historia Animalium, De Partibus Animalium, De Generatione Animalium" (Venice, 1476) is the first edition of the Latin translation by Theodorus Gaza. That doodling was an ancient habit is evident from the contemporary patterns made in the margins by one Angelus Calydonius, who signed his name on p. 110, September 17, 1491.

It is noteworthy that nearly all the authors of biological books in the sixteenth and seventeenth centuries were medical men. Their works, for reasons that may be inferred, were frequently dedicated to dignitaries of the Church—cardinals and popes. Their subject was primarily the human body, though with references, not always acknowledged, to the bodies of animals. A beautiful copy of Galen's works in six volumes ("Omnia Quae Extant Opera ex Secunda Juntarum Editione") is in the Library. It was printed at Venice in 1550. But Galen's day was passing, and the professor no longer would read chapters from his works to his students. Independent accounts of the human body, based on dissection, were appearing. The first of these, and the most valuable book in the library, is "De Humani Corporis Fabrica" by Andreas Vesalius, first edition, printed at Basle in 1543. The second edition, 1555, is in its original tooled leather binding, with metal clasps, by Johannes Mechler, 1557. Prof. Cole never tired of showing these two magnificent folios to his visitors.

Giulio Casserio (1552–1616)

A valuable French book of the period is Estienne's "La Dissection des Parties du Corps Humain" (Paris, 1546). Its beautiful binding is in red morocco, tooled gold. Thomas Vicary's "The English Man's Treasure, with the true Anatomie of Man's Bodie" (Black Letter, London, 1596) is a transcript of a fourteenth-century manuscript, and is therefore pre-Vesalian. Only two other copies of this edition were known to Prof. Cole—one in the Bodleian and the other in the Surgeon-General's Library in Washington. The first Englishman to make a systematic study of natural history was the physician Edward Wotton, who graduated, as Harvey did, at Padua. His book "De differentiis Animalium" (Lutetiae Parisiorum, 1552) is a fine copy bound in limp vellum.

The chair of anatomy at Padua was filled during the sixteenth and seventeenth centuries by Vesalius, Columbus, Fallopius, Fabricius, Casserius, Spigelius and Veslingius. Vesalius has already been mentioned, and works by Columbus, Fabricius, Casserius and Veslingius are represented. Of the three works of Fabricius (Harvey's teacher) the most important is "Tractatus Quatuor" (Francofurti, 1624) which contains the section "De Venarum Ostiolis", and describes, without understanding of their function, the valves of the veins. The beautiful publications of Casserius, the pupil and understudy of Fabricius, include a fine copy, with portrait, of his "De Vocis Auditusque Organis Historia Anatomica", bound up with "De Auris Auditus Organi Historia Anatomica" (Ferrariae, 1601, 1600) and by his "Pentaestheseion, hoc est De Quinque Sensibus Liber" (Venetiis, 1609). Both works describe the sense organs of animals as well as those of the human subject.

The study of aquatic animals interested physicians at this period. Pierre Belon, called by Casey Wood "the founder of modern ichthyology", published "L'Histoire Naturelle des Estranges Poissons Marins, avec la Vraie Peincture et Description du Dauphin" (Paris, 1551). This, the first edition, is very rare. But the finest early work on fishes is Salviani's "Aquatilium Animalium Historiae" (Romae, 1554), famous for its eighty-one full-page copper-plate engravings of fishes and marine creatures by Nicolas Béatrizet. Rondelet's "Libri de Piscibus Marinis" (Lugduni, 1554–55), with its cruder but more numerous woodcuts of all kinds of marine creatures, is in its original tooled leather binding, with metal clasps.

An interesting, rare and beautiful volume, the provenance of which was described by Prof. Cole in his "Obiter Dicta Bibliographica"[1], is Carlo Ruini's "Dell'Anotomia et dell'Infirmita del Cavallo" (Bologna, 1598). This is the first complete work on the anatomy of an animal, and was plagiarized by Snape and others.

We come now to the seventeenth century, "the most constructive and significant period in the history of modern biology". The whole animal kingdom is reviewed in the eleven publications of Aldrovandus, ranging from 1613 to 1668. Four books by Severino include a fine copy of his "Zootomia Democritaea", with portrait (Noribergae, 1645). Six works of Walter Charleton, with a copy of the first edition of his "Onomastikon Zoicon" (Londini, 1668). Eight works by Redi, who in his "Esperienze intorno alla Generatione degl' Insetti" (Firenze, 1668), "successfully attacked by experiment the universal belief in

spontaneous generation". Nine works by Jan Swammerdam, whose "Ephemeri Vita of afbeeldingh van's Menschen Leven" (t'Amsterdam, 1675), is the first edition of a superb monograph on the may-fly. Two volumes of Samuel Collins's "Systeme of Anatomy, treating of the Body of Man, Beasts, Birds, Fish, Insects and Plants" (London, 1685), the first treatise on comparative anatomy by an Englishman, and with an extremely fine engraved portrait by Faithorne. Edward Tyson's "Orang-outang [that is, chimpanzee], sive Homo sylvestris, or the Anatomy of a Pygmie compared with that of a Monkey, an Ape and a Man" (first edition, London, 1699) is signed "The Author's gift, June 9, 1699".

The seventeenth century was the century of William Harvey. Prof. Cole did not possess a first edition of Harvey's "De Motu Cordis et Sanguinis Circulatione"; but he had copies of all the facsimile productions and translations down to Dr. Franklin's translation of 1958. In this work Harvey put the last stone on an edifice, the foundations of which had been built by many other workers, but in his work on generation in animals ("Exercitationes de Generatione Animalium"; first edition, Londini, 1651) he was a pioneer, and the book was described by Prof. Cole as the "work of a sadly puzzled human being". The Elzevir edition (Amstelodami, 1651) was T. H. Huxley's copy.

A remarkable feature of the seventeenth century was the foundation of learned societies in the great capitals of Europe, and the participation of groups of investigators in research. Though the series of the *Philosophical Transactions of the Royal Society* is not complete, the earliest and rarest volumes and also long runs are in the library. The fifteen volumes of the German "Academia Naturae Curiosorum" (Frankfurt und Leipzig, 1670–90) is a fine series, bound in the original vellum. Of the Danish publication, "Acta Medica et Philosophica Hafniensia", associated with Thomas Bartholin, 1671–79 (all issued), published 1673–80, only two other complete copies were known to Prof. Cole. Vol. 1 bears the signature of Clopton Havers, M.D. The Amsterdam Society was short-lived, and Prof. Cole was unable to purchase a copy of its *Proceedings*, so he had a facsimile edition of a hundred copies of "Observationes anatomicae selectiores Collegii privati Amstelodamensis" (Amstelodami, 1667–73), printed in the Typography Department of the University of Reading in 1938. Of the "Académie des Sciences" in Paris, there are the two volumes, large folio, of C. Perrault's "Mémoire pour servir à l'histoire naturelle des Animaux" (Paris, 1671 and 1676), and a magnificent copy of the 1676 volume, handsomely bound in red morocco, with the royal fleur-de-lis tooled in gold, and showing on the title-page Louis XIV and his minister Colbert attending a meeting of the Academy in the Royal Library at Versailles in 1671. The Italian Society, Accademia del Cimento, is in Richard Waller's English translation, entitled "Essayes of Natural Experiments made in the Academie del Cimento" (London, 1684).

Returning to the Royal Society, there are the works of many of the original Fellows. Robert Boyle's six works include the complete works in five volumes printed in 1744; this set was formerly the property of the Boyle family, and bears the bookplate of the Earl of Cork and Orrery. Thomas Willis is represented by six works, including the first edition of "Cerebri Anatome", 1664, Robert Hooke by seven works, including the first and second editions of the "Micrographica" (1665 and 1667 respectively), Nehemiah Grew by six works, including his "Anatomy of Vegetables Begun" (1672), "The Anatomy of Plants" (1682) and "The Comparative Anatomy of Stomachs and Guts Begun" (1681) and Marcello Malpighi by seven works, a most valuable and complete set, including the "Opera Omnia", which Prof. Cole made perfect by buying three copies and binding the three volumes in one, with the portrait and the life (Londini, 1687, 1686 and 1697). There are fine copies of "Dissertatio epistolica de Bombyce" (first edition, Londini, 1669) and "Dissertatio epistolica de Formatione Pulli in Ovo" (first edition, Londini, 1673). Among the eight works of Antoni van Leeuwenhoek, the Dutch draper who, working in isolation, had to trust to his own observation and judgment, are the Dutch edition of his works in four volumes (Leyden and Delft, 1684–1718), the Latin edition (Lugd. Bat., 1722), the select works in four volumes (1798–99 and 1800–07) and the current "Collected Letters of Antoni van Leeuwenhoek, vols. 1–5 (all published to date) (1939–57).

The eighteenth century was a period of great activity by numerous major and minor investigators in all branches of biology, particularly in the attempt to understand the functions of the organs of animals. So physiology went side by side with anatomy. The microscope, too, was coming into use, thus enabling the minutest parts of animals to be examined. Museums were founded, and great skill was shown in preserving, injecting and otherwise exhibiting whole animals or their parts. There was a great accumulation of factual knowledge in preparation for the Darwinian era in the following century. Only a few of the outstanding works can be mentioned, chosen to illustrate the diversity of treatment and subject. "Oeuvres anatomiques de M. Duverney" (two volumes, Paris, 1761) contains reprints of his beautiful and detailed studies on the sinuses of the dura mater, on the structure, function and diseases of the ear, and the first accurate account of the solar plexus. Spallanzani's seventeen works include his "Essay on Animal Reproductions", translated from the Italian of 1768 (London, 1769), the forerunner to his conclusive experiments on the origin of life, described in his "Opuscula di Fisica Animale et Vegetabile" (first edition, two volumes, Modena, 1776). Comprehensive faunistic works were beginning to appear, one of the earliest being O. F. Müller's "Zoologica Danica" (third edition, Havniae, 1788–1806) with many illustrations of marine animals. It was published posthumously. The learned Swiss Albrecht von Haller, physician, physiologist, bibliographer and poet, has twenty-one entries in the catalogue. As a bibliographer his work is indispensable to a historian of biology, and the library contains his "Bibliotheca Botanica" (2 vols., Tiguri, 1771–72), "Bibliotheca Chirurgica" (2 vols., Bernae, 1774–75), "Bibliotheca Anatomica" (2 vols., Tiguri, 1774–77) and "Bibliotheca Medicinae Practicae" (4 vols., Bernae and Basiliae, 1776–88). It is said that Haller's library contained 13,500 volumes and that he wrote more than five hundred scientific papers. Prof. Cole was a great admirer of John Hunter, and his famous Museum, on which he planned his own Museum at the University of Reading. Hunter was not a diligent writer, and there are only six books of his in the Library; but there are nearly forty entries of references to him and his work. A complete publication of John Hunter's works in four volumes was published in London, 1835–37. John's elegant brother

William was a renowned lecturer. Prof. Cole had two sets of manuscript notes of his lectures (c. 1770), one of 280 pages, testifying to the appreciation and diligence of two of his students.

The nineteenth and twentieth centuries have produced a voluminous literature in every branch of zoology. The Library contains beautiful monographs in many languages. It is also rich in biographical and bibliographical works. Among the latter may be mentioned the Index Catalogue of the Library of the Surgeon General's Office, U.S. Army, Washington, which Prof. Cole used constantly. The first, second and third series, in 16, 21 and 10 volumes respectively, are present. Of the modern transcriptions and translations there are the works of Leonardo da Vinci, namely, "Codice sul volo degli Ucelli" (first edition, Rouveyre, Parigi, 1893), with the perfect facsimile of the original, "Dell'Anatomia, Fogli A" (Parigi, 1898), "I Manoscritti di Leonardo da Vinci, della Reale Bibliotheca di Windsor. Dell'Anatomia, Fogli B" (Torino, 1901), half the series (13 volumes) of the "Feuillets inédits" (Paris, 1901) and "Quaderni d'Anatomia", six volumes (Christiania, 1911–16), a truly noble collection, as most of these are limited editions.

The above selection can give only a meagre idea of the richness and scope of this wonderful library. Prof. Cole's wishes were that it should not be dispersed, but should be available *in toto*, as it has been in the past, to scholars in the history of biological thought. This happy outcome has, we believe, been achieved by its acquisition by the University of Reading.

[1] Macmillan and Co., Ltd., 1944; reprinted with corrections, 1949.
[2] *J. Hist. Med. Yale*, **13**, 1 (1958).

OBITUARIES

Prof. W. N. Thomas, C.B.E.

Prof. W. N. Thomas, professor of engineering in University College, Cardiff, during 1928–50, died on November 24.

His initial engineering training was as pupil and assistant of the Borough Engineer at Crewe.

During 1910–15 he lectured at the Universities of Nottingham and Birmingham. He then saw war service with the Royal Engineers in France and Flanders. He returned to university life at Birmingham and afterwards at Oxford, where he was chief assistant to Prof. Jenkin for five years and received the degrees of M.A. and D.Phil. He left Oxford to become principal assistant at the Building Research Station at Garston, and three years later, in 1928, he was appointed professor of engineering at Cardiff, a post in which he remained until his retirement in 1950.

His time at Cardiff was broken by war service. In 1939 he became scientific adviser to the Ministry of Home Security, and an expert on the effects of bombs on structures. He visited Moscow in 1941 and led a team of specialists to Japan in 1945 to report on the atom bomb damage at Nagasaki and Hiroshima. In 1946 his war work was recognized by the award of the American Medal of Freedom and he was made C.B.E.

On retirement from the University of Wales, Norman Thomas became president of the South Wales Institute of Engineers, and presided over the difficult negotiations which led to its amalgamation with the South Western Society of Mining Engineers. He continued his association with the Home Office and became chief scientific adviser for Wales on civil defence. He relinquished this appointment only a year ago. He was also active in the affairs of the Institution of Civil Engineers and made a number of journeys abroad on its behalf.

His academic interests were well spread. Norman Thomas was an authority on the effect of frost on materials, and he published many papers on the mechanical properties of materials. He was author of a well-known text-book on surveying, and completed a further revision within a year of his death. He also wrote a book on bridges and was interested in military bridging. While at Cardiff he succeeded in having constructed an addition to his somewhat modest laboratories, and he lived long enough to take part in a token demolition of the wooden walls which were his academic inheritance; and to see the demolition proper commence. His colleagues greatly regret that he did not survive a few more years to see the splendid new laboratories which will replace them.

In personal characteristics, Norman Thomas was modest and retiring, but his quietly expressed opinions were well received by his colleagues, and he had an unusual capacity for inspiring other people's affection.

C. Gurney

Dr. Pierre A. Chappuis

The Swiss zoologist Dr. P. A. Chappuis, who died at Zurich on July 9, 1960, was for many years assistant director of the Institut de Spéologie at Cluj, Rumania, where he also acted as Swiss Consul. At the time of his death he was attached to the Centre National de la Recherche Scientifique as director of research and assistant director of the Laboratoire Souterrain de Moulis (Ariège), France.

He was born on September 5, 1891, at Saint-Cloud, Seine-et-Oise, France, where his father was assistant director of the International Bureau of Weights and Measures. Educated in Switzerland, he obtained his doctorate at the University of Basle in 1920; his thesis on the fauna of the subterranean waters in the neighbourhood of Basle was prepared under Prof. F. Zschokke. The following year he joined the staff of the recently created Institut de Spéologie at Cluj and in 1927 he succeeded R. Jeannel as assistant director. He remained at his post when, following the Treaty of Vienna, a large part of Transylvania was ceded to Hungary and his Rumanian colleagues went into exile (1940–45). E. Racovitza, the founder and first director of the Institut, died in 1947, and political events compelled Chappuis to leave Rumania in 1948.

Until serious thyroid trouble put an end to strenuous activity, he was a great traveller. In 1921 he and A. David explored the region of the White Nile. In 1929 he joined R. Jeannel and Ch. Alluaud in an expedition to the Sahara. In 1931 he and Alluaud crossed the Sahara and collected extensively in the regions of the Upper Volta and Ivory Coast.

In 1932–33 he accompanied Jeannel and Arambourg on their expedition to the River Omo and climbed Mt. Elgon. He and his colleagues visited hundreds of caves in little-known or unexplored parts of Transylvania, notably in the Bihar Mountains, and in old Serbia.

A fuller account of his life, together with a complete list of his numerous scientific publications, will appear in a memorial part of the *Annales de Spéléologie*. He wrote the third volume of Thienemann's "Die Binnengewässer", entitled "Die Tierwelt der unterirdischen Gewässer" (Stuttgart, 1927). In the same year he also contributed the section on Anaspidacea to Kükenthal and Krumbach's "Handbuch de Zoologie". He was an authority on various groups of freshwater and subterranean Crustacea, especially harpacticids, bathynellids and isopods. His discovery, in 1942, of the true habitat of *Bathynella* and other minute animals that occur sporadically in wells, springs or caves opened a new field of investigation. Since then numerous new or little-known interstitial animals, freshwater and marine, have been discovered in many parts of the world. Many of the Crustacea, including a European species of Mystacocarida, were described by Chappuis and his collaborators and his last years in France were exceedingly productive ones.

In 1922 he married Heidie Spinnler, of Basle; their son, Pierre, resides in Basle, and their daughter is married to an English architect.

Isabella Gordon

Dr. Gertrude L. Elles, M.B.E.

The doyen of women geologists, Dr. Gertrude L. Elles, emeritus reader in the University of Cambridge and honorary Fellow of Newnham College, died at Helensburgh on November 18, in her eighty-ninth year. She entered Newnham as a scholar in 1891 and remained, with few interruptions, resident in Cambridge until a few weeks before her death.

She obtained first-class honours in Part II of the Natural Sciences Tripos (Geology) in 1895 and for the next few years was associated with Lapworth at Birmingham and with Törnquist and others in Sweden. This initiated the work on graptolites for which she is so widely known, and she soon commenced with Dame Ethel Shakespeare the preparation of the Palæontographical Society's monograph on British graptolites under the editorship of Prof. Lapworth. The ten descriptive parts of this classic work appeared with extraordinary regularity between 1901 and 1914 and established her position as a world authority on these fossils. Of a dozen or so smaller publications on them, the most significant was her "Graptolite Faunas of the British Isles" (Geologists' Association, 1922), which has exercised a profound influence on subsequent work on their faunal succession and evolution.

Nevertheless, Dr. Elles's interest in fossils was pre-eminently that of the field geologist and stratigrapher, and, concurrently with her work on the graptolites, her investigations on Lower Palæozoic stratigraphy in Wales and the Welsh Borderland resulted in several papers on the geology of areas around Ludlow, Conway, Bala and Builth. Relatively late in her career, she turned to a completely new field in 'hard-rock geology', and jointly with Prof. C. E. Tilley published a notable contribution on metamorphism in relation to structure in the Scottish Highlands.

Dr. Elles was closely associated with the Sedgwick Museum (or its forerunner, the Woodwardian Museum) for nearly seventy years. Her standing in the Department of Geology was formalized by her appointment, under the new statutes, to a University lectureship in 1926; ten years later, she became the first woman reader in the University. Few who have graduated in the Cambridge Department of Geology during this long period but were influenced by her enthusiasm as lecturer or supervisor; and many were drawn to Cambridge as postgraduate students to work under her direction. She inspired great affection among her colleagues, and as a teacher she will be remembered for her vitality, forthright manner and clarity of exposition.

She held the degree of doctor of science of Dublin and of Cambridge. When the Geological Society of London extended its fellowship to women in 1919, she was one of the small but distinguished group at once admitted, and she was in the same year the first woman to receive the Society's Murchison Medal. She was president of Section C (Geology) of the British Association in 1923.

O. M. B. Bulman

NEWS and VIEWS

Royal Society: New Gold Medal

The Council of the Royal Society has accepted an offer by the Trustees of the Leverhulme Trust Fund to mark the occasion of the Royal Society's Tercentenary by the award of a gold medal. Under the terms of the offer, the medal is "for award by the Society, every three years, to the individual who in the opinion of its Council shall have made the most significant contribution in the field of pure or applied chemistry or engineering including chemical engineering. The medal, which will be in gold, will be accompanied by a monetary award of five hundred pounds". The Council has made the first award of the medal to Sir Cyril Hinshelwood, who retired as president of the Royal Society on November 30, for his outstanding contributions to physical chemistry.

Officers

Sir Howard Florey, president of the Royal Society, has appointed the following vice-presidents for the year ending November 30, 1961: Sir Alexander Fleck, treasurer of the Royal Society, formerly chairman of Imperial Chemical Industries, Ltd.; Sir Lindor Brown, biological secretary of the Royal Society, Waynflete professor of physiology in the University of Oxford; Sir William Hodge, physical secretary of the Royal Society, Lowndean professor of geometry and astronomy in the University of Cambridge; Sir Patrick Linstead, foreign secretary of the Royal Society, rector of the Imperial College of Science and Technology, London; Prof. T. M. Harris, professor of botany in the University of Reading; Dame Kathleen Lonsdale, professor of chemistry at University College, London.

The Gas Council's London Research Station : Mr. G. U. Hopton

Mr. G. U. Hopton has been appointed the new director of the Gas Council's London Research Station at the Fulham Laboratories. He was senior research chemist of the North Thames Gas Board from 1952, until the retirement of Dr. R. H. Griffith last August, when he became acting director. Mr. Hopton gained a first-class honours degree in chemistry at Oxford and the same year joined the Gas Light and Coke Company. In 1936 he was awarded the MacNab Medal of the Institution of Chemical Engineers and in 1945 gained the H. E. Jones Medal of the Institution of Gas Engineers. From 1950 until this year he was chairman of the board of examiners of the Institution of Chemical Engineers. Mr. Hopton has also been vice-president of the Institution. He is a member of the board of studies in engineering of the National Council for Technological Awards.

British Museum (Natural History) : Appointments

The Principal Trustees of the British Museum have appointed Dr. F. C. Fraser, keeper of zoology, and Mr. J. E. Dandy, keeper of botany, to be deputy chief scientific officers in the British Museum (Natural History). They have also appointed Mr. J. P. Doncaster to be keeper of the Department of Entomology in the British Museum (Natural History), in succession to Dr. W. E. China, who will retire on January 31 ; and Dr. J. P. Harding and Mr. J. F. Perkins to be deputy keepers in the Departments of Zoology and Entomology, respectively.

Mr. Doncaster, who has been a deputy keeper in the Department of Entomology since December 1955, was educated at Leighton Park School, Reading, and St. Catharine's College, Cambridge. After some years on entomological work at the Plant Virus Research Station, Cambridge, he entered the British Museum (Natural History) in 1937, to take charge of the newly formed Exhibition Section. During the War he was seconded to the Agricultural Research Council to work on the aphid vectors of potato viruses, returning to the Museum in 1945 to begin the re-designing and reconstruction of war-damaged exhibits. In 1951 he joined the staff of the Department of Entomology to take charge of the collections of Homoptera (Sternorhyncha) and Thysanoptera, and to reorganize the collections of Aphididae. His published works have dealt mainly with the systematics of aphids and their role as virus vectors.

Dr. Harding was educated at Torquay Grammar School, University College, Exeter, the University of Cincinnati, and King's College, Cambridge. Prior to joining the staff of the British Museum (Natural History) in 1937, Dr. Harding worked for a year as assistant naturalist in the Ministry of Agriculture and Fisheries. Dr. Harding is a carcinologist specializing in the Entomostraca, on which he has published many papers in journals of learned societies.

Mr. Perkins was educated at Newton College and the Imperial College of Science and Technology (Royal College of Science), London, graduating in 1932 and being awarded the Forbes Medal. He entered the British Museum (Natural History) in 1933, where he has worked on the parasitic Hymenoptera, and in particular Ichneumonidae. During the War he was seconded to the Ministry of Agriculture, where he was associated with some of the early trials of D.D.T. against crop pests. His published works have been concerned with the general classification of the Ichneumonidae, and revisions of groups of this family particularly in relation to the British fauna.

Exhibition of The Institute of Physics and The Physical Society

If one function of science is to enable us to rationalize our sense perceptions, we need instruments and apparatus to help us perceive ; the coincidence of the pointer and scale is of profound importance. As our scientific knowledge ramifies and increases in complexity, demands upon apparatus for precise measurement and manipulation become more exacting. The manner in which this demand has been met so far will be clearly demonstrated at the annual exhibition to be held by The Institute of Physics and The Physical Society at The Royal Horticultural Society's Halls, Westminster, London, S.W.1, from January 16 until 1 p.m. on January 20. Lectures will be given by : Mr. F. S. Burt (Admiralty Research Laboratory) on "Hydrodynamics Research", on January 17 ; Dr. G. E. R. Deacon (National Institute of Oceanography) on "The Physics of the Ocean", on January 18 ; and Dr. F. P. Bowden (Laboratory for the Physics and Chemistry of Solids, Cambridge) on "Some Physical Problems in Travelling at Supersonic Speed", on January 19. These lectures will be at 5.45 p.m. The prize-giving of the Craftsmanship Competition will take place in the lecture theatre on January 19 at 3 p.m. The Society has published a *Handbook of Scientific Instruments and Apparatus* (price 6*s*., plus 2*s*. postage), which is a guide to the exhibits. It is also a reference book on recent developments in the design of physical apparatus ; it provides a list of manufacturers and of recent scientific books. As a means of planning one's visit to the Exhibition, the handbook seems indispensable. Further information can be obtained from The Institute of Physics and The Physical Society, 47 Belgrave Square, London, S.W.1.

Institute of Information Scientists

A full syllabus for education in information work has now been prepared by the Institute of Information Scientists ; it has also been approved by Aslib. Courses on "Collecting and Communicating Scientific Information" based on this syllabus have been arranged at the Northampton College of Advanced Technology, St. John Street, London, E.C.1, to start on January 24, and will comprise evening classes twice a week during two years. The Institute will hold (external) examinations, based on the syllabus, for the Certificate of the Institute, and will be set in two groups, at the end of 1961 and 1962, respectively. Details of the course may be obtained from the College, and other information from the honorary secretary of the Institute, J. Farradane, "Torran", Crofton Road, Orpington, Kent.

Forest Research and Training Institute for Latin America

A focus for forest research and training in Latin America has been acquired with the permanent establishment—following four years on a temporary basis—of the Latin American Forestry Institute for Research and Training at the Forestry School of the University of Los Andes, Merida, Venezuela. A multilateral agreement has been reached between the Food and Agriculture Organization, under the aus-

pices of which the Institute will operate, the Government of Venezuela, and other interested member nations of the Organization. So far, Venezuela, Guatemala, Nicaragua, Panama, Peru, Colombia, France and the Netherlands have become party to the agreement. Several other countries are expected to join in the near future. The aims and functions of the Institute are fourfold: it will carry out research projects, particularly those of broad interest and of an applied nature which would contribute to the conservation, utilization and development of Latin American forest resources; it will organize forestry training courses in line with future demands for both Government and private foresters in Latin America; it will gather, classify and disseminate forestry information; and it will promote regional co-ordination of research by informing member Governments of forest research being carried out in Latin America.

Health Science Man-power in the United States

A survey of the man-power needs in health science for the United States is to be carried out by the Federation of American Societies for Experimental Biology. Objectives of the survey will include: (1) an assessment of the present supply and demand for scientists in each of about twelve basic health fields; (2) projections of such supply and demand for the next 10–20 years; and (3) recommendations as to how the increased demand for these scientists may be met. Dr. John T. Cowles, director of educational planning for the health professions at the University of Pittsburgh, is director of the project. Dr. Lowell S. Levin has joined Dr. Cowles's staff in Pittsburgh to serve as full-time associate director of it. Some of the data being sought include: number of persons now employed, salary-levels, space requirements, current student enrolments, research expenditures, training programme income, instructional costs and student aid. The over-all project, which will require three to five years to complete, will be expanded from microbiology to include most, if not all, of the health science fields. The experimental phase of the project is being supported by an 87,000 dollar grant from the National Institutes of Health. The University of Pittsburgh has been selected to carry out the survey because of its available personnel and electronic computer facilities which will be required.

Educational Film Centre, Ltd.

A new organization, the Educational Film Centre, Ltd., has recently been founded. Its directors are Maurice Goldsmith, formerly science editor at Unesco, John Halas and Joy Batchelor, animation film producers, and Dr. Roger Manvell, formerly director of the British Film Academy. The objects of the Centre include promoting the production of educational films, and specially of science-teaching films, sponsorship of research into the most useful forms and techniques for instructional films and their best use in the class-room, and publication of pamphlets and film-strips associated with the films produced and with the research projects undertaken by the Centre. The Centre is in close consultation with the Educational Foundation for Visual Aids. The initial aim is the production of a planned series of science-teaching films designed specifically for use in secondary modern schools. These films will be made in collaboration with representatives from science teachers' associations, who have already undertaken to give advice and help. The Centre is to operate from Lysbeth House, 10a Soho Square, London, W.1.

Wallace and Darwin

More information about the theory of organic evolution put forward jointly by A. R. Wallace and Charles Darwin is contained in recent publications. In a recent issue of the *Proceedings of the Linnean Society of London* (171, Part 2, 139; 1960), Prof. C. F. A. Pantin introduces the essay prepared by Wallace in 1855 which served to stimulate Darwin to join him, and led to the publication of their famous paper of 1858. Mr. Tom Harrisson, curator of the Sarawak Museum, in a forthright article in the "Proceedings" of the Centenary and Bicentenary Congress of Biology held in Singapore during 1958, is concerned chiefly with Alfred Russel Wallace and a letter he wrote to Charles Darwin from Ternato in the Celebes in 1858. The author contrasts the background of these two men—Darwin, the son of wealthy parents, and Wallace, born of humble parentage. It was the Ternate letter, Mr. Harrisson claims, that forced Darwin's hand, for on its receipt "he had to speak or forever lose his place in the priorities of the new human thought".

In the fourth of his articles on Darwin's Notebooks on the Transmutation of Species, Sir Gavin de Beer deals with the Fourth Notebook, concerned with the period October 1838–July 10, 1839 (*Bulletin of the British Museum* (*Natural History*), Historical Series. Vol. 2, No. 5. Pp. 151–183. (London: British Museum (Natural History), 1960.) 15*s*.). Darwin's Fourth Notebook on Transmutation of Species was written after he had read Malthus's "Essay on the Principle of Population" on October 3, 1838. Considering the importance of the influence which Malthus's book was thought (even by Darwin himself in later life) to have exerted on Darwin's work and ideas, it is significant that he devoted so little space to Malthus in his Notebook which he wrote immediately after reading his essay. The reason is that Darwin had already and independently thought out the principle of selection of favourable variations and seen the possibility that the transmutation of species might be explained by its means. What Malthus gave Darwin was evidence of the rigorousness of selection and of the inevitability of widespread mortality. To this concept Darwin introduced the notion of extinction as the extreme case of depopulation and the notion of variation; and he showed that he was well aware that this would lead to results very different from those which Malthus thought he had achieved.

Darwin's Original Papers

In 1942 the Pilgrim Trust and the Darwin family presented to the University Library, Cambridge, and to Down House, the original papers of Charles Darwin. Those intended for Cambridge arrived at the Library after the War and have been bound in volumes representing as closely as possible the papers in the order in which Darwin left them. A detailed hand-list of the papers was made in 1955 by Miss R. V. C. Bailey and Miss J. S. Gosse for the use of scholars working on the papers in the University Library. This hand-list has now been published in view of its general interest, together with a subject index and an index of correspondents compiled by Miss G. M. B. Tyrer (Pp. 72. Cambridge: At the University Press, 1960. 10*s*.).

Preservation of Wild Animals in Asia

MUCH has been written in recent years on the threat of extermination hanging over much of the fauna of Africa and on possible methods of averting it. Less has been heard about the equally pressing danger to the fauna of other parts of the world. The timely appearance of a report on some animals of the Middle East and southern Asia which are threatened with extermination entitled "A Look at Threatened Species", by L. M. Talbot (*Oryx*, the Journal of the Fauna Preservation Society, **5**, Nos. 4 and 5, 157; May 1960), is therefore particularly welcome. The report is based on information collected during an extensive journey of reconnaissance in 1955 extending from Libya to Formosa. The investigation was concerned principally with the past and present status of the three species of Asiatic rhinoceros, the Indian lion, the Arabian oryx, and the Syrian wild ass; but the report also deals with many other species. It is a most interesting and well-written document that considers in some detail such matters as wild-life habitats and land-use, and gives an account of such reserves and parks as there are in the territories visited. One of the largest problems facing conservation is the lack of trained personnel. In most former 'Colonial' territories conservation and management were carried out by men from the colonizing country. When they departed, the country was left with very few people with any training at all in conservation matters. Nevertheless, although in many places there has been, and still is, much wanton destruction, a real effort is being made in others at conserving what is left. This report will strengthen the hand of the International Union for the Conservation of Nature in its efforts to help those concerned in preventing the final extermination of a number of interesting species of animals.

Geomorphological Abstracts

As a guide to the very scattered literature of interest to geomorphologists, a small journal of abstracts, in an attractive format, has recently been produced by a group of British geographers. In the first two numbers, for June and September 1960, abstracts are given of 136 recent papers, mainly works in English-language periodicals (No. 1; June 1960. Pp. 23. Published four times a year in March, June, September and December). At present some 120 journals are covered, and it is hoped to increase this number as circumstances permit. *Geomorphological Abstracts* will be published quarterly, and can be obtained from K. M. Clayton, Department of Geography, London School of Economics, Houghton Street, London, W.C.2, price 16*s*. per annum, post paid.

Canadian Museum Publications

THE National Museum of Canada has added to its guide-books two publications which should be well received by visitors to the Museum. The first, "Canadian Atlantic Sea Shells", by E. L. Bousfield, is an illustrated account in English of shell-bearing invertebrate animals commonly encountered on the beaches or in shallow waters of the Canadian Atlantic coast from the Strait of Belle Isle and the St. Lawrence estuary on the north to the ocean coasts of Newfoundland and Nova Scotia and the Bay of Fundy in the south. Altogether, 150 sea shells are described (Pp. v+72, 13 plates. Ottawa: Queen's Printer, 1960. 1 dollar). The second, "Les Mammifères du Canada", by Austin W. Cameron, contains descriptions in French of the mammals of Canada, each description being accompanied by a line drawing prepared by John Crosby (Pp. 89. Ottawa: Queen's Printer, 1960. 50 cents).

Scientific Instrument Manufacturers' Association

THE Scientific Instrument Manufacturers' Association has published a booklet, "Careers in the Scientific Instrument Industry", providing a well-illustrated summary of what a new entrant into the industry can expect to find and what his work and training may be like (Pp. 20. London: Scientific Instrument Manufacturers' Association of Great Britain, 1960). The launching of the booklet has been encouraged by the rapidly increasing number of apprenticeship certificates issued by the Association's members under their endorsement scheme in recent years. In 1958, 52 certificates were endorsed, 64 in 1959, and 82 in the first nine months of 1960, or at a rate double that of 1958. The endorsement ensures that certain standards of training with the appropriate equipment are the foundation of the apprentice's certificate, and provide him with the start to a career in the scientific instrument manufacturing industry. The booklet can be obtained from the Scientific Instrument Manufacturers' Association, 20 Queen Anne Street, London, W.1.

Information on Atomic Energy in Britain

UNDER the title "A Guide to Information on Atomic Energy in Britain", the Central Office of Information has issued an illustrated select list of British books, periodicals, reports, films, photographs and display materials, describing the latest nuclear developments in the United Kingdom, which was originally compiled for specialized and prestige distribution overseas (Pp. 64. London: H.M. Stationery Office, 1960. 5*s*. net). The list was prepared with the co-operation of the United Kingdom Atomic Energy Authority. The material is arranged under visual aids; general publications; serial publications; the power programme and nuclear engineering; radioisotopes; nuclear physics; and radiation hazards and protection. Books for the specialist and for the general reader are distinguished. There are also further lists of the Atomic Energy Authority's unclassified publications (including a list of depository libraries in the United Kingdom), and of publications covering legislation in the United Kingdom and co-operation with other countries.

Restoration of a Silk Flag

THE *Museums Journal* of November, in an article by Mr. Harry Wandrus, describes an economical method for the treatment of deteriorating silk flags. The flags were the property of the State of Ohio and most of them were of 'weighted' silk, that is, they contained chlorides or sulphates of magnesium, tin, iron or aluminium. Unfortunately, these chemicals have the effect of causing an early deterioration of the silk, which then crumbles and powders. The essence of the process is to soften the old flag with water and carefully assemble the parts in their correct positions on a flat table ready for a white silk chiffon backing. This backing should be neatly arranged over the flag and fastened with a 5 per cent solution of polyvinyl alcohol. The flag will then hang well in a vertical position. Full details of the process are given.

Atomic Weight of Silver

Silver is an important secondary standard of atomic weights, the values of these for many elements having been determined with reference to silver. The natural element contains two isotopes, 107 and 109, and the average atomic weight has been accepted as 107·880, on the chemical scale. A mass-spectrometer investigation by W. R. Shields, D. N. Craig and V. H. Diebeler, of the Bureau of Standards, Washington, is reported in the October issue of the *Journal of the American Chemical Society* (p. 5033). It was found that no variation in abundance ratio occurred in six specimens of natural silver, but a seventh, from Cobalt, Ontario, showed a significant deviation. The values of the atomic weight found were, on the physical scale ($^{16}O = 16$) 107·9028 ± 0·0013, on the chemical scale (O = 16) 107·8731 ± 0·0020, and on the proposed unified scale ($^{12}C = 12$) 107·8685 ± 0·0013. It is seen that the chemical value differs appreciably from 107·880.

Binocular Depth Perception

One of the difficulties in studying depth perception is to isolate the different factors that contribute to the three-dimensional effect, and in particular to separate the binocular from the monocular cues. A highly original method of achieving this separation has been developed at the Bell Telephone Laboratories by B. Julesz, and, under the heading "Binocular Depth Perception of Computer-Generated Patterns", the subject is discussed in a recent issue of *The Bell System Technical Journal* (39, 1125; 1960). In this method the two pictures to be viewed in a stereoscope consist of a random arrangement of black, white and grey dots. These two micropatterns are similar in dot distribution except for a central area where, for example, a square-shaped region is given a uniform horizontal displacement. Each pattern by itself has a completely random appearance, but when viewed in a stereoscope, the parallax produced by the lateral displacement causes the central square region to appear either in front of or behind the surround, depending on the direction of displacement. Several interesting features emerge from this method of presentation in which monocular cues are eliminated. For example, several seconds or even minutes may elapse before the depth effect is perceived, presumably while the eye and brain are searching the pattern and assimilating the information being presented to the subject. When the viewing is repeated, the perception-time may be shortened as the process of learning facilitates recognition of the pattern. The effect of varying the contrast of the pattern, its sharpness, different perturbations of the pattern and the introduction of a monocularly recognizable pattern have all been studied to see how far the depth effect is enhanced, although just what is meant by depth enhancement is not immediately obvious.

The Night Sky in January

Full moon occurs on Jan. 1d. 23h. 06m. U.T., new moon on Jan. 16d. 21h. 30m. and full moon on Jan. 31d. 18h. 47m. The following conjunctions with the Moon take place: Jan. 1d. 17h., Mars 8° N.; Jan. 20d. 05h., Venus 0·6° N.; Jan. 26d. 13h., Aldebaran 0·3° S.; Jan. 28d. 07h., Mars 8° N. Mercury will be visible as an evening star near the end of the month, setting about one and a half hours after the Sun. Venus is an evening star, and is favourably placed for observation. Venus sets at 20h. 00m., 20h. 35m. and 21h. 15m. on January 1, 15 and 31, respectively, about four hours after the Sun. During the month its distance from the Earth decreases from 83 to 63 million miles, the visible portion of the apparent disk from 0·639 to 0·499, and its stellar magnitude from −3·7 to −4·1. Mars is above the horizon all night on January 1, and sets at 7h. 10m. and 6h. 5m. on January 15 and 31, respectively. Mars is very conveniently situated for observation. It is at a distance of 61 million miles from the Earth on January 15, and its stellar magnitude is −1·0. Jupiter and Saturn are too close to the Sun for observation. Occultations of stars brighter than magnitude 6 are as follows, observations being made at Greenwich: Jan. 7d. 5h. 09·8m., 59 Leo. (*R*); Jan. 24d. 19h. 57·2m., +11° 445 (*D*); Jan. 26d. 23h. 59·6m., 318 B. Tau. (*D*). *D* and *R* refer to disappearance and reappearance, respectively. The Quadrantid meteors are active on January 3, but conditions are unfavourable for observation. The Earth is at perihelion on January 2, its distance from the Sun being 91·4 million miles.

Announcements

Dr. Detlev Bronk, president of the Rockefeller Institute, has been elected an honorary member of the Brazilian Academy of Science and has been awarded an honorary doctorate of the University Brazil.

Dr. Amyand David Buckingham has been awarded the Harrison Memorial Prize for 1959 in consideration of his work on solvent effects on molecular spectra and the theory of pressure-induced spectra. Dr. Buckingham is at present in the Department of Inorganic Chemistry, University of Oxford.

Dr. H. Harold Hume, of Gainsville, Florida, has been awarded a gold Veitch Memorial Medal by the Council of the Royal Horticultural Society for his work on horticulture and in particular for his work in connexion with camellias.

The Systematics Association are arranging a meeting on "Multivariate Methods in Taxonomy" to be held in the Department of Botany, the University of Southampton, during April 24–25, 1961. Further information can be obtained from Dr. J. M. Lambert, Department of Botany, University of Southampton.

"Radio Techniques and Space Research" will be the theme of next year's British Institution of Radio Engineers' Convention which will take place during July 5–9, 1961, at the University of Oxford. The Convention will contain at least six sessions, provisionally entitled: "Review of the Role of Radio Techniques in Space Research"; "Satellite Engineering Problems"; "Extra-terrestrial Measurements"; "Telemetry"; "Techniques in Radio Astronomy"; "Satellites in Terrestrial Communications". The Convention Committee has invited offers for papers, and these should be addressed to the British Institution of Radio Engineers, 9 Bedford Square, London, W.C.1.

The Chemical Society, Burlington House, London, W.1, has published "Periodicals in the Chemical Society Library", giving the complete periodical holdings in the Library (Pp. 48. London: The Chemical Society, 1960. 5*s*. post paid). This will be useful not only to members of the Society but also to librarians and users of chemical literature generally.

ORGANIZATION OF SCIENTIFIC RESEARCH IN LATIN AMERICA

A REGIONAL meeting to discuss the organization of scientific research in Latin America was held during October 3–7 in the Central University of Venezuela, Caracas. The meeting was arranged by the Unesco Science Co-operation Office for Latin America, under the patronage of the Ministry of Education and the University of Venezuela. Heads of national research councils, rectors of universities, deans of science faculties and senior scientists from Argentina, Bolivia, Brazil, Chile, Colombia, Cuba, Ecuador, Mexico, Peru, Uruguay and Venezuela had been invited to attend, and the World Health Organization and the National Academy of Sciences of the United States sent observers. The meeting was held at the Central University.

The president of the Republic, Sr. Rómulo Betancourt, spoke briefly at the opening ceremony, welcoming the delegates with the assurance that they would find in the Venezuela of to-day complete freedom for the development of scientific research. Among other speakers were Dr. Establier, head of the Unesco Science Co-operation Office for Latin America; Dr. Rafael Pizani, Minister for Education, and Dr. Francisco De Venanzi, rector of the Central University.

The meeting elected Dr. Venanzi as its chairman. The working sessions were opened by a review of the general tendencies of scientific research in Latin America by Prof. Bernardo Houssay, president of the Council for Scientific and Technical Research of Argentina. This paper provoked a valuable discussion which formed a background for the other papers presented at the meeting.

The paper by Dr. David Stitchkin, rector of the University of Concepción, Chile, on funds available for scientific research, with special reference to equipment, was the one which caused least discussion, since the meeting was unanimous in concluding that the amounts at present available were notably insufficient. It was recommended that Governments should accept as a target the allocation of 2 per cent of the national budget for scientific research.

During the discussion of the paper by Dr. Mario Gonzalez (Cuba) on the basic and applied sciences, it became clear that the meeting did not wish to emphasize the distinction between the two, and preferred to think of science as a whole, recommending that firms in industry and agriculture should strengthen their links with research and teaching institutions in view of the profound effect which science has on modern commercial life.

Prof. Houssay introduced the question of national research councils, which exist in only a very few Latin-American countries, including the Argentine, Brazil and Mexico. The other Governments of the region were recommended to give serious thought to the advantages of establishing research councils, and Unesco was asked to provide information to Governments about the functions and possible types of organization of research councils.

Dr. Marcel Roche, director of the Venezuelan Institute of Scientific Research, presented a paper on the career of the research worker. He analysed and compared the careers offered by the research councils of Argentina, France and Venezuela. It was thought that the arrangements adopted by the Argentine and Venezuelan Councils, whereby their staffs were able simultaneously to accept teaching posts, was valuable in the Latin-American scene, since it made maximum use of the small scientific man-power available, and kept the research worker in touch with universities and youth. This subject was very closely related to the next one discussed, the shortage of teaching and research personnel in Latin America, which was introduced by Dr. Rodolfo Talice, dean of the Faculty of Science in the University of Montevideo.

One of the topics of greatest interest at the meeting was the paper by Dr. Paulo Sawaya, dean of the Faculty of Philosophy of the University of São Paulo, on the results of the adoption in certain universities and institutions of the full-time system. Most universities and research organizations permit their staffs to hold one or more posts outside the organization, and the virtues and vices of this system form one of the most hotly debated subjects in scientific circles in Latin America. The meeting recommended that Governments and universities should establish the full-time system as the ideal in research and higher teaching posts, and that they should provide salaries and working conditions adequate for this purpose.

Unesco has encouraged and assisted the formation of regional research centres, such as that for mathematics in Buenos Aires, and of groupings of research centres, such as the Latin-American Council for Cosmic Radiation. Dr. Ismael Escobar, head of the Cosmic Ray Physics Laboratory at Chacaltaya, Bolivia, introduced this topic, the discussion of which led the meeting to call on Unesco to continue its efforts in this field, so that teams of research workers might be brought together, animated by the desire to achieve Latin-American unity in research.

Dr. De Venanzi analysed the attitudes of Governments and of public opinion towards scientific research problems in Latin America. Though much improvement has occurred in the past few years, a great deal remains to be done. The meeting felt that the Press, radio and television had a paramount part to play, and hoped that ways could be found to establish training in scientific journalism. In this connexion, delegates were happy to note the complete and accurate coverage of the meeting in the Caracas Press.

In Latin America, fellowships tenable in more scientifically developed areas will, for some time to come, provide the principal source of higher training for research workers in many fields. Dr. E. Beltran, Under-Secretary for Forestry and Game Resources in Mexico, discussed the subject in all its aspects. An increase in the number of fellowships granted by governments and other agencies was recommended, as well as greater emphasis on instruction in foreign

languages, since language deficiencies frequently prevented full benefit from fellowships. The paper by Dr. M. A. Garrido Malo, dean of the Science Faculty in San Marcos University, Lima, was on a closely allied subject—exchange of scientists between Latin-American countries. Unesco was asked to publish its *Boletin* with greater frequency, so that news of courses, special facilities, visits of foreign scientists, etc., might be more rapidly disseminated, and all laboratories were exhorted to use to the utmost the facilities offered.

The subject of scientific documentation in Latin America was introduced by Dr. Manuel Sadosky, vice-dean of the Science Faculty in the University of Buenos Aires. A lengthy discussion led to a series of recommendations: that Unesco should make a survey of scientific journals in Latin America, with the object of formulating a plan for rationalizing them; that the Mexican Scientific Documentation Centre should make its services more easily available to scientists in other countries; that more national scientific documentation centres should be established, that Governments should grant free postal services to scientific journals; and that financial help might be given to a select number of journals.

The final subject on the agenda was a review of Unesco's science programmes, particularly that of the Latin American Science Co-operation Office in Montevideo. Dr. Establier described proposals to be debated at the general conference of the Organization held in Paris in November, and also outlined a series of long-term projects which might be undertaken by the Montevideo office. Among these was one for an International Andean Year, during which teams of specialists in different fields would attack the problems of this vast mountain chain which so profoundly affects living conditions in those countries through whose territories it runs. Another proposal was for a full-scale operation towards rationalizing scientific journals. A third would deal with the problems of the unproductive areas of the seas, lakes and rivers of the continent. At the end of this discussion Dr. Establier received the warm congratulations of the meeting, both for the way he has directed Unesco's science activities in Latin America, and for the organization of the meeting.

B. A. Houssay

INTERNATIONAL OPERATIONAL RESEARCH

From its War-time start in Britain, operational research has become an international movement. The first conference on this basis was held three years ago at Oxford. It led to the establishment of the International Federation of Operational Research Societies, of which the chief architect was Sir Charles Goodeve, who was elected its first general secretary. Under his guidance the Federation continues to expand. Great Britain has acted as the Federation's first 'foster' society, an office which shortly passes to America; and France was designated 'host' society for the second of the triennial conferences, called at Aix-en-Provence during September 5–9. The Société Française de Recherche Opérationnelle has discharged its duties with grace and efficiency, at a conference presided over by its own president, Prof. G. T. Guilbaud.

The Second International Conference on Operational Research had the object of surveying the whole scene, and its organizers limited the attendance to 350 delegates. Representation at the national level was strictly allocated according to the sizes of national societies, who themselves had the invidious task of selecting their delegations from long lists of applicants. The programme was determined in outline by a committee which appointed leading workers in this field as sponsors of more than twenty sessions; each sponsor was responsible for designing and presenting his own session, for inviting some papers, for selecting others from among those submitted freely, and for taking the chair at his session at Aix-en-Provence. These arrangements led to a more tightly controlled conference than is customary at this level, and were intended to produce a coherent pattern, high-quality material, and authoritative discussion.

Delegates arrived from no less than twenty-three countries; and although two-thirds of them were drawn from America, Britain and France, there was plenty of evidence that significant operational research efforts have been mounted in at least a dozen more countries. The extent of the applications of operational research to-day might have surprised those who first embarked on the attempt to apply the methods of science to the strategic problems of war. But there are strategic problems in all managerial situations, and eighty papers presented at Aix-en-Provence attest to the fact that operational research has by now penetrated into the intricacies of most of them.

The conference opened with a plenary session dealing with the methodological aspects of operational research. Whispers were heard complaining that once again the subject had succumbed to mere introspection, to a self-conscious preoccupation with its own anatomy. Most scientists in the field of operational research would agree, however, that a subject devoted to scientific investigation of the methods of management has something to gain from the examination of its own. Thus it proved. The session brought out clearly the fact that the goals of an organization are various and not necessarily consistent, that they are constantly changing, and that they reflect a process of learning to survive by adaptation. It follows that crude statements of aim, such as 'maximum profit', which have often been naïvely emulated by equally crude scientific models, are inadequate. There are always ways of estimating and comparing organizational efficiency, but they require experimentation on possible models and not merely experimentation within some plausible model. The sensitivity of a top-level decision to explicit assumptions about likely circumstances is itself an object of study; but its sensitivity to implicit assumptions about goals is still more important. For this reason the model of an adaptive organism adjusting itself to its environment is valuable, and much was said about attempts to use scientific descriptions of biological learning and

goal-seeking processes as models of business behaviour.

The first group of parallel sessions then set out to discuss two general problems and five areas of application. Six papers were presented dealing with new mathematical methods: they concerned especially the use of the theory of graphs in studying supply networks, and various treatments of the problems of sequential decision. Another group of papers dealt briefly with the question of controlling production, but without much originality. The areas of application were: transport, oil, power, steel and local government. Examples of transport operations by road, rail and air were given in which operational research had solved the strategic problems of scheduling fleets, and of choosing between alternative policies, for example, in overhaul, cargo carried and design. The oil industry put forward studies of the flow of traffic and the problems of stock-holding, and of the selection of investments. There were three papers in the session on power dealing with the operation of nuclear reactors, and studies were also presented of the central planning of an atomic energy industry and of an electricity distribution system. From the steel industry there were discussions of the control of spare parts and of the unloading of ore, and a study of track times. The local government session was devoted to city and land-use planning, and included a consideration of a city as a teleological mechanism generating its objectives intrinsically.

The formal sessions were broken up by a series of discussion groups. These were often extremely spirited conversations. They dealt, for example, with the organization of operational research, with its future needs, and with education—the latter subject leading to a considerable clash about the role of the universities and possible syllabuses. One group discussed economic decisions that could involve loss of life, and two more the allied techniques of operational gaming and of simulation. There was a meeting on operational research for under-developed countries. But perhaps the most remarkable session of all dealt with operational research at the top-decision-making level in industry. This involved some of the most forceful interchanges of the whole conference, and continued all day—although it was billed to last for the morning only. The discussion was too frank, too fast, and too heated ever to reach the printed proceedings: an added reason for the agreement of the main protagonists that this day alone had been worth the journey to France.

At this point, the second plenary session was held, to consider the relationship between operational research and computers. It was held that the existence of these machines, considered properly as logical engines rather than as calculators, had enabled operational research to forge new tools. Those discussed were a computer language for the automatic programming of simulations, which had quartered the time required to prepare an intricate programme, and a computer analysis of the field of heuristic decision processes. Secondly, however, computer applications themselves received benefit from operational research assistance. In this area, a mathematical model was presented for the optimal storage and retrieval of information, and an operational research survey was made of the factors involved in the introduction of on-line computers for controlling production.

The second series of parallel sessions included two general problem areas, those of inventory control theory and mathematical programming, and descriptions of operational research in three applied fields; mining, war and national government. Inventory theory is by now an extensively explored topic, but seven more contributions were made dealing with particular aspects of the problem. There were nine contributions also to the subject which began as linear programming, but which has long since been extended to such cases as the linear programme under uncertainty, various non-linear models, and quadratic programming—all discussed here. In the mining session, a general account of operational research at the National Coal Board in Britain was presented, as well as papers on the determination of optimal mining limits and the study of pit haulage. The presentation of military applications was necessarily restricted, but the relevance of the theory of games was discussed, as well as problems of communications and stock-holding policy. The growing effort in operational research at the national level was well expressed in various papers dealing with the whole economic system of a country, with national planning in India, with the co-ordination of investment in transport, and with social decision-making.

The third and last plenary session was concerned with human factors. Hospitals were considered as viable organisms, according to an adaptive model reminiscent of the methodological talk at the beginning of the week, and there were other papers on the human element in decision systems. A doleful note was sounded at the end of a strenuous conference by the report of some interesting research into the acceptance of solutions. Teams involved in business gaming were presented with the mathematically optimal strategy for their game after they had become implicated in the play; but in no case could any means be found by which they could be persuaded to use it.

The conference was closed with an address by Sir Charles Goodeve, who characteristically eschewed the usual valedictory platitudes. Instead, he issued a considerable challenge to the movement to play a bigger part in the social field, by bringing operational research to bear on the problem of evaluating alternative policies by developing a measure for human happiness. His address is reported *in extenso* elsewhere[1], and it will be interesting to see what fruit it has borne when the Third International Conference on Operational Research meets in Oslo in 1963, when the Norwegian society is expected to be host.

The proceedings reported here were set in the familiar context of receptions, tours, committee meetings, and arbitrarily arranged discussion groups —some of which lasted until dawn. Enthusiasm was a keynote of the assembly, as befits a young endeavour, and it was quite evident that much important operational research is being done that does not reach print. The first reason for this is an occupational hazard of the work: the advisors of management cannot readily air the secrets of institutional policy in public. But the second reason seems to be the convention that reports of operational research ought to include wads of respectable mathematics, whereas in real life the mathematics involved in operational research are often trivial.

Much was said at Aix-en-Provence publicly to acknowledge this fact, and to emphasize again the approaches for which operational research has always stood: scientific method, with its insistence on

measurement, experimentation and logical inquiry; interdisciplinary collaboration; above all, the seeking of usable insights rather than the production of abstract and possibly sterile formulæ. This reaffirmation should do something to increase the supply of good literature on operational research. It will certainly encourage those whose intentions are concentrated whole-heartedly on the essentially practical task of helping policy-makers reduce the influence of chance on the outcomes of their decisions.

STAFFORD BEER

[1] See *Nature*, **188**, 180 (1960).

BIOLOGICAL STRUCTURE AND FUNCTION

IN 1956, the International Union of Biological Sciences decided to set up a Biochemistry Section Committee which would be a co-ordinating committee between itself and the International Union of Biochemistry. One of the main functions of this Committee is to make recommendations to both Unions for international symposia, embracing subjects in which both biochemistry and the biological sciences are combining to produce a rapidly expanding sphere of knowledge; on this basis the subject "Biological Structure and Function" was chosen for the first joint symposium which was held at the Wenner-Grens Institute, Stockholm, during September 12–17. About 150 scientists attended the symposium, at which 72 members from 11 different countries presented invited papers to the seven sections of the symposium.

The first section was on macromolecular structure and function (chairman, A. Tiselius). Dr. J. C. Kendrew (Cambridge) described with great clarity the work which he has carried out on the elucidation of the structure of myoglobin, and Dr. P. A. Albertsson (Uppsala) discussed a new technique for separating macromolecules by partitioning them in aqueous two-phase systems containing polymers such as dextran and polyethylene glycol. This method has great possibilities in, for example, purifying viruses and separating different sized fragments of disintegrated mitochondria and chloroplasts. The second day, devoted to the subject of microsomes and protein synthesis, was memorable for a masterly discussion of the endoplasmic reticulum by the chairman, Dr. K. R. Porter (New York), which culminated in a series of electron micrographs which clearly demonstrated the existence of this structure in plant cells. In the more biochemical section the emphasis was on the role of ribonucleoprotein particles in protein synthesis.

Mitochondrial structure and function were discussed on the third day (chairman, O. Lindberg). At the moment, two subjects appear to polarize the interest of the various investigators studying the relationship between mitochondrial structure and function. One is the phenomenon of mitochondrial swelling. It seems, however, that different lines of approach are being followed by various laboratories. On one hand, swelling and shrinking are related to the transition of mitochondria from one to another well-defined functional state, for example, from a state of full respiration where the mitochondria are found to shrink, to a state in which the respiration is limited by the absence of phosphate acceptor and where the mitochondria are inclined to swell. On the other hand, mitochondrial swelling is considered to be the result of a more complete change of the normal structure with a loss of most of the mitochondrial functions; the inhibition of the swelling by different agents or the reverse of swelling by way of a 'contraction' of the swollen mitochondria are followed as means to define the physiological implications of the phenomenon. The other topic, to which numerous papers were devoted, was the mechanism of electron transport and phosphorylation in the diphosphopyridine nucleotide–flavin region of the respiratory chain. The interest in this point was raised both because of the finding that in the presence of flavo-substrates a reversal of electron transfer could take place by way of reactions requiring high-energy phosphate, and because of the suggestion that this phenomenon could be related to an adenosine triphosphate activation of succinate oxidation, which on the other hand is not inhibited by uncoupling agents. It seems that work on these two reactions may provide a good deal of information on the question of the synthesis and utilization of the high-energy compounds during the operation, both in the forward and backward directions, of the terminal part of the respiratory chain.

In the morning session on chloroplast and chromatophore structure and function (chairman, T. W. Goodwin), electron micrographs of chromatophores isolated from both purple and green photosynthetic bacteria were shown by Drs. A. W. Frenkel (Minneapolis) and J. A. Bergeron (Upton, N.Y.), and Dr. M. D. Kamen (Waltham) speculated ingeniously on the primary act of photosynthesis. Later in the day Dr. D. I. Arnon (Berkeley) described his latest investigations on photosynthesis in cell-free systems of *Chromatium* and Dr. Birgit Vennesland (Chicago) gave her views on photophosphorylation with particular reference to the possible role of a 'nascent formate' intermediate, recently postulated by Warburg. The developmental physiologists discussed intact cellular structure and function on the morning of the fifth day, under the chairmanship of Prof. J. Runnström. The topics discussed ranged from 'mitosis', when Dr. D. Mazia described it in terms of a multitude of individual reactions co-ordinated into a fixed time pattern, to electron microscopy studies on ciliary and flagellar movements (B. Afzelius).

In the afternoon an important session on polysaccharides (chairman, G. Blix) included lectures by Dr. S. Hestrin (Jerusalem) on "The Growth of Saccharide Macromolecules" and by Dr. A. Dorfman (Chicago) on "The Acid Mucopolysaccharides of Connective Tissues", and concluded with another important paper on technique from Prof. Tiselius's laboratory, when Dr. P. Flodin described the gel-filtration method for separation of oligosaccharides.

The final session on specific membrane transport and its adaptation (chairman, B. D. Davis) produced further lively discussion, and the closing half-hour

was a salutary reminder that, in spite of the mass of information imparted during the week, our knowledge of the relationship between structure and function is still fragmentary—apparently two currently irreconcilable views are held on the mechanism of amœboid movement.

The arrangements made by Prof. O. Lindberg and his colleagues in the Wenner-Grens Institute for this symposium were superb, and, thanks to their hard work and genius for organization, everything moved smoothly. Furthermore, the Wenner-Gren Foundation generously supported the symposium financially, and without its aid its existence would have been highly problematical. As could well be imagined, remembering the nationality of our hosts, the social side of the symposium was not neglected and highlights included a banquet in Skansen and a visit to a performance of Pergolesi's "The Music Master" at the famous seventeenth-century Drottlingholm Theatre. A specially delightful feature was that all foreign delegates spent at least one evening in a Swedish home.

The International Union of Biochemistry and the International Union of Biological Sciences are truly grateful to their Swedish hosts for arranging the first joint symposium, and hope that this Stockholm symposium will be the forerunner of similar international symposia based on fruitful co-operation between biochemists and biologists from all nations. It is hoped to publish the proceedings of the symposium in book form in the near future.

T. W. Goodwin

POWDERS IN INDUSTRY

Sir Alexander Fleck, the president of the Society of Chemical Industry, in opening the first symposium of the Surface Activity Group of the Society of Chemical Industry held during September 29–30, commented on the many imperfectly understood problems of physics and physical chemistry relating to industrial applications of powders, and referred to the largely empirical nature of many old-established manufacturing processes involving powders. "In recent years, however", he said, "application of the growing body of knowledge on the surface properties of powders has led to striking improvements in both processes and products and will undoubtedly lead to further developments, including the foundation of new industries".

In the words of Dr. M. G. Fleming, chairman of the organizing committee, the subject of powders was chosen for the symposium because so many industries make or use powders either as raw materials, intermediates or final products, many manufacturers being unaware of developments in otherwise unrelated industries which could assist them. The programme, he said, was planned to "sketch in the background and define the fundamental terms; next examine the particular requirements and problems of a diverse group of industries, and, finally, correlate these specialized applications, underlining their contiguity and exposing the shortcomings in our basic knowledge of the subject".

A laudable aim, but the event left the impression of unevenness and there were marked swings in the discussion from academic argument concerning, for example, the meaning of surface tension, to some severely practical details of powder processing. This was perhaps the inevitable result of covering so wide a field, theoretical and practical, in a relatively short time, but there was great value in bringing together so much material, the sequential grouping of which proved admirable.

Thirty-one authors contributed in the four sections, which covered "Principles of Production", "Properties of Powders", "Properties of Powders utilized in Industrial Fields", and "Principles of Application and Problems for the Future". There were seven sessions, each with its own chairman and an introducer, who presented the papers as a group on behalf of the authors.

The papers of the first two sections concerned powder production by milling and by controlled precipitation, and the recovery and processing of natural powders; the surface energy of solids, adsorptive properties of powders, adhesion between particles, particle size, shape and surface area, and the electrical and flow-properties of powders. These together provided an excellent general background against which to study papers in the industrial section covering pigments and fillers in paints, printing inks, rubber and plastics (including a paper on whiting); powders as used in building cements, ceramics, bituminous road materials, cosmetics, detergents, pharmaceuticals, pesticides, paper, drilling fluids, and explosives; and problems of mineral dressing, soil mechanics, powder metallurgy, and coal and smokeless fuel production.

The papers revealed extensive basic research, much of it in relation to particular practical problems, and there was much evidence of considerable skill in process manipulation based on experience and technical appraisal of the products. In a brief report it is, however, impossible to give details of the numerous problems in powder production, manufacture and use, calling for further attention, or on which more fundamental knowledge would appear to be needed.

On the production side there were many references to the inefficiency of powder handling machines, particularly grinding mills (better understanding of energy relationships could lead to new methods of grinding), and to the unknowns in present methods of powder drying and powder size classification processes.

From the papers covering numerous industrial uses it was clear that while many of the outstanding problems are specific to particular industries, work in one field is not always being fully utilized in others. On the other hand, techniques of study successful in one industrial context can be unsuited to different materials and applications. For example, methods of particle-size determination adequate for size-ranges down to, say, one micron, giving useful information for particular applications, may be quite unsuitable for the much smaller particles sometimes involved in other types of product.

There is also the problem of how to measure effective or operative particle sizes, in particular

environments, for while knowledge of the geometry of individual primary particles is valuable, frequently the operative unit is an agglomerate, flocculate or cluster of particles. Often information about what is happening to the constituent particles in an industrial product has to be deduced from the rheological, mechanical or other properties of that product, there being an inadequacy of methods for directly observing the behaviour of particles within the mass.

In many industrial applications much more information is required about the interplay of particle and particle; about the behaviour of particles distributed in gas or liquid in various concentrations in relation to the behaviour of single particles in the same environments; also about the nature of the bond between such particles and the surrounding medium, not only in the static product, but also during the various processes involved in its application or use and subsequent life.

What is the present state of basic knowledge about powders? Here the conference was greatly assisted by the surveys made in the final section, with their collected information on well-established physico-chemical principles, and on the forces known to operate in dry or wet powders. Careful consideration of such information in relation to particular systems could, it was suggested, indicate possible means of controlling the mechanical behaviour of powders or suspensions.

There were calls for more information on the fundamentals of powder formation, and of the flow properties, internal packing, etc., of powders; also on the molecular origin of electrostatic charges, adhesion between particles, and the cohesion of the mass. There were indications of the kinds of basic work needed to improve understanding of the factors affecting dispersion and flocculation of particles in solid/liquid systems, involving the inherent surface chemistry of solids, adsorption, chemisorption, etc., and the fine-structure of particles relative to methods (for example, adsorption methods) of measuring 'surface area'. Recent rapid advances in microscopy, and in electron microscopy in particular, were noted, with the suggestion that microscopists should seek still further to improve their techniques.

To summarize, the symposium brought together a mass of material, fundamental and practical. It revealed the 'zones of ignorance' and the kind of investigations required for further advance. Individual powder-using industries can be expected to continue the search for new basic knowledge pertinent to the solution of their own problems, but their task would be facilitated by more extensive fundamental studies at the universities and in central research organizations. The field having been so well surveyed, it would be well for the participants to meet again in two or three years time to examine progress.

S. H. Bell

COMPOSITION OF MILK IN THE UNITED KINGDOM

Until quite recently the quality of milk has been judged mainly on its fat content, but in the past thirty years an increased understanding of the principles of nutrition has made it clear that the most important constituents of milk are not the fats but the proteins, minerals and water-soluble vitamins which are, of course, to be found in the non-fatty solids portion of the milk. Research in the past decade has indicated that since 1930 or so in England and Wales the content of solids-not-fat in milk, and therefore the nutritive value of the milk, has tended to decline slightly but significantly. For many herds the solids-not-fat content of the bulked milk has been maintained well above the presumptive legal limit of 8·5 per cent, but for an undesirably high proportion of herds it has frequently been below it.

These facts led in 1958 to the appointment by the Minister of Agriculture, Fisheries and Food, the Minister of Health and the Secretary of State for Scotland of an interdepartmental committee under the chairmanship of Dr. J. W. Cook to consider the composition of milk and to recommend any legislative or other changes that might seem desirable. The Committee's report has now been published*. Various members of the Committee formed three panels, which considered medical and animal husbandry aspects, and the freezing-point (Hortvet) test for detecting the presence of added water in milk. The reports of all three panels are published with the main report.

The Committee points out that great progress has been made in past years in improving the hygienic quality of milk; its members rightly believe that similar progress should now be made in improving its nutritive quality. To that end they have put forward some twenty-three main recommendations. They suggest, among other things, that the presumptive minimum legal standard of 8·5 per cent for the solids-not-fat content of milk should be abolished and that price penalties should ultimately be imposed when milk contains 8·4 per cent and less. They recommend also that milk-recording associations should provide means for testing the milk of individual cows for solids-not-fat as well as for fat.

There will certainly be many problems to solve before all the main recommendations made in the report can be implemented, and some breed societies and other bodies concerned will feel that the recommendations go much too far, while others will inevitably think that they do not go far enough. However that may be, it is clear that the Committee has done a very thorough job and that the adoption of its recommendations should have the desired effect of arresting and even reversing any tendency there may be for the nutritional quality of milk in the United Kingdom to decline. The report contains a wealth of information on the subject of the composition of milk and will long remain a most valuable and reliable work of reference on that subject.

* Milk Composition in the United Kingdom: Report of an Interdepartmental Committee. Pp. iii+92. (Cmnd. 1147.) (London: H.M. Stationery Office, 1960.) 5s. net.

A THEORY OF THE MECHANISM OF ACTION OF CREATINE PHOSPHOKINASE

By Dr. B. R. RABIN and D. C. WATTS
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As part of a programme of investigations on the evolution of biological catalysts, extensive work has been carried out in this Department on the mechanism of action of creatine phosphokinase from rabbit muscle. This enzyme catalyses the reaction:

$$\underset{\text{adenosine triphosphate}}{\text{ATP}} + \underset{\text{creatine}}{\text{Cr}} \rightleftharpoons \underset{\text{adenosine diphosphate}}{\text{ADP}} + \underset{\text{creatine phosphate}}{\text{Cr—P}}$$

It has been shown that the rate of formation of creatine phosphate (forward reaction) is maximal at *p*H 8·8–9·0, whereas the *p*H optimum for the formation of adenosine triphosphate (reverse reaction) is 6·0–7·0, depending on the buffers used. The enzyme requires the presence of magnesium(II) or manganese(II) ions, and it has been suggested[1] that a metal adenosine tri- or di-phosphate complex is the active substrate for the enzyme. Dixon[2] has stated, without giving any evidence, that there is reason for believing that the enzyme-substrate combination involves the formation of a thiol-magnesium phosphate complex. The available evidence favours neither of these hypotheses[3].

Creatine phosphokinase has two reactive thiol groups per mole[4], and alkylation of these with iodoacetate or iodoacetamide, destroys the enzyme activity. The kinetics of this reaction have been studied by measuring both the loss of enzyme activity and the production of iodide ions (using a silver/silver iodide electrode). These investigations have shown that only one of the SH groups is associated with the enzyme activity.

The reaction of the enzyme with iodoacetate and iodoacetamide as a function of *p*H is shown in Fig. 1. It can be seen that, over the *p*H range studied, the reaction-rate is independent of *p*H. This surprising finding is explicable if both thiols are hydrogen-bonded to other groups. The effects of various components of the substrate system on the rate of alkylation of the thiol groups are shown in Table 1. The results were obtained by following the production of iodide ions, which measures the reaction of both thiol groups. Since only the thiol group at the active site is affected by modifiers, and the two thiol groups react at nearly the same rate (unpublished work from this Department), the kinetic effects on the active site are approximately twice those shown in Table 1. The significant effects have been confirmed by following the loss of activity. The simultaneous presence of all substrates and magnesium(II) causes considerable protection of the enzyme against reaction with both iodoacetate and iodoacetamide; in the absence of the metal, there is no significant protection.

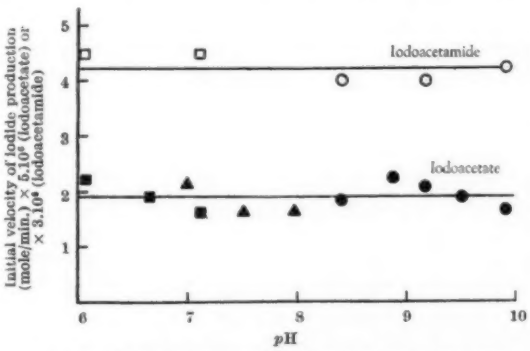

Fig. 1. Effect of *p*H on the rate of alkylation of creatine phosphokinase. The reagent and protein concentrations were, respectively: for iodoacetate, 0·0005 *M* and 0·42 mgm./ml.; for iodoacetamide, 0·0001 *M* and 0·4 mgm./ml.
Buffers: □, ■, diethylmalonic acid/sodium hydroxide; ▲, *tris*-(hydroxymethyl)aminomethane; ○, ●, boric acid/sodium hydroxide. Temperature, 25° C.; ionic strength, 0·12

Table 1. Effects of Substrates on the Initial Rate of Alkylation of Creatine Phosphokinase at 25° C. in Borate Buffer *p*H 9·0 at Ionic Strength 0·12

Substrate added	Relative initial rate of iodide production (rate in absence of added substrate = 1·0)	
	Iodoacetate	Iodoacetamide
Nil	1·00	1·00
Equilibrium mixture + Mg(II)*	0·65	0·66
Equilibrium mixture†	0·91	1·05
Mg(II) (0·001 *M*)	1·01	0·97
ATP (0·001 *M*)	0·96	1·00
ATP (0·001 *M*) + Mg(II) (0·001 *M*)	0·81	1·00
ATP (0·001 *M*) + Cr (0·01 *M*)	0·96	1·07
Cr (0·01 *M*)	1·03	1·07
Cr (0·01 *M*) + Mg(II) (0·001 *M*)	1·01	0·93
ADP (0·001 *M*)	1·00	1·00
ADP (0·001 *M*) + Mg(II) (0·001 *M*)	1·27	1·01

* Obtained by allowing 0·001 *M* adenosine triphosphate (ATP), 0·01 *M* creatine and 0·001 *M* magnesium(II) to proceed to equilibrium before adding the alkylating agent.

† Approximate only. Consisted of the following mixture: adenosine di- and tri-phosphate, 0·0005 *M*; creatine phosphate, 0·0007 *M* creatine, 0·005 *M*.

The effects of ionic strength on the reaction of the enzyme with iodoacetate and iodoacetamide are shown in Fig. 2. The decrease in reaction-rate with increasing ionic strength observed with iodoacetate could be due to an electrostatic interaction between the carboxyl group and a positive locus on the enzyme. Thus it is evident that the effects of the various substrate components on the reaction of the protein with iodoacetate will be difficult to interpret because of this complication.

The failure of the metal ion, alone or in the presence of some of the substrate components, to protect the thiol group at the active site against alkylation argues against the metal being bound to the thiol.

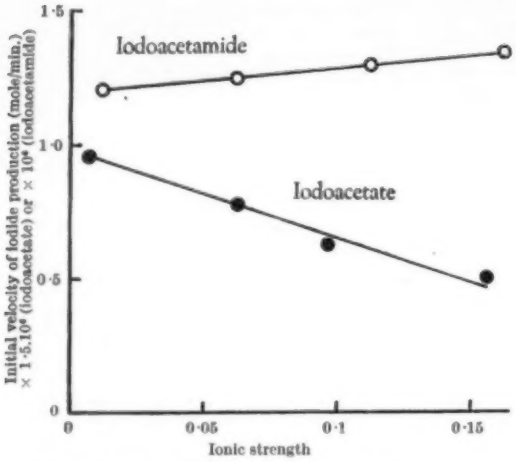

Fig. 2. Effect of ionic strength on the rate of alkylation of creatine phosphokinase. The reagent and protein concentrations were, respectively: for iodoacetate, 0·0005 *M* and 0·57 mgm./ml.; for iodoacetamide, 0·0001 *M* and 0·4 mgm./ml. Temperature, 25° C.; *p*H 9·0 (boric acid/sodium hydroxide)

The high degree of specificity possessed by the enzyme for both guanidine and purine components[1] indicates that these groups are bound at specific sites on the protein. If it is assumed that the thiol at the active site is hydrogen-bonded, perhaps to an imidazole residue, it is possible to visualize a mechanism for this enzyme which fits most of the data. The hydrogen-bonded thiol-imidazole system can be represented by Fig. 3. We suggest that interaction of the guanidine group with the thiol occurs only when both substrates and the metal are simultaneously present. The forward reaction can be represented by Fig. 4. In this the dashed lines are the bonds being formed or broken. The reaction proceeds by a synchronous set of bond re-arrangements, the rate-limiting step in which would be the simultaneous rupture and formation of the O—P and N—P bonds. The hydrogen-bond re-arrangements should occur with much greater facility than the nucleophilic displacement process at the phosphorus atom.

Fig. 3

The function of the metal in the forward process is to orientate the phosphate groups and assist the reaction by partial neutralization of the charges on the phosphate oxygen atoms. If the electronic tendencies shown in Fig. 4 are taken to completion, the species indicated in Fig. 5 are obtained. At high *p*H values a proton will ionize from adenosine diphosphate, probably while this is still attached to the enzyme surface, driving the reaction in the forward direction. Tautomeric re-arrangement, independent of *p*H, regenerating the thiol-imidazole hydrogen bond system shown in Fig. 3, would occur prior to dissociation of the substrates from the enzyme surface. The formation of adenosine triphosphate

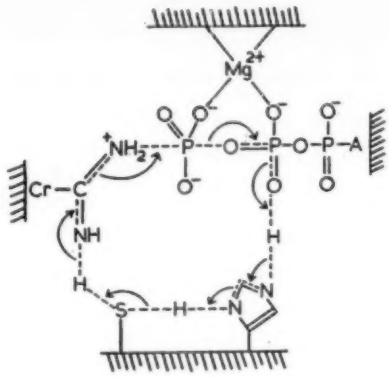

Fig. 4

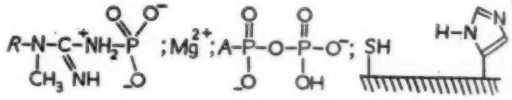

Fig. 5

would occur by complete reversal of the pathway of the forward reaction. Addition of a proton to enzyme-bound adenosine diphosphate is needed to trigger the reverse reaction, and this accounts for the lower *p*H optimum of the reverse process. The reverse reaction can be visualized as proceeding via the enzyme-substrate complex indicated in Fig. 6. Several tautomeric variants of this formulation are possible. A prototropic re-arrangement of thiol-imidazole would give the intermediate shown in Fig. 4 and the reaction would proceed by a counter-clockwise set of bond-shifts, the reversal of those in Fig. 4. The function of the metal in the formation of adenosine triphosphate is to hold the attacking phosphate of adenosine diphosphate near the phosphorus atom of creatine phosphate and assist the nucleophilic displacement at this atom by partial neutralization of the charges on the oxygen atoms.

It can be shown, by the use of models, that the proposed mechanism is stereochemically feasible. We have shown in the diagrams interaction of the sulphur with the guanidino group and interaction of

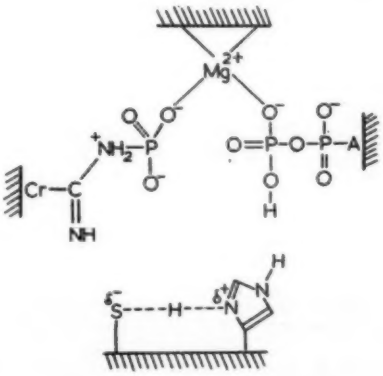

Fig. 6

the imidazole with a phosphate oxygen. It is, of course, possible that these sites of interaction are reversed without affecting the essential basis of the mechanism. This reaction is an interesting illustration of the principle of conjugate requirements[8]; in this instance the acid/base groups on the protein surface involved with attacking and departing groups are hydrogen-bonded together.

The mechanism we propose can easily be extended to other phosphokinases, and it is hoped that it will act as a guide and stimulus to further work on phosphate transfer systems.

We are indebted to the Central Research Fund, University of London, for a grant for the purchase of the automatic titration equipment used in these experiments. One of us (D. C. W.) thanks the Agricultural Research Council for a personal grant.

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A POSSIBLE SPECIFIC CHROMOSOME ABNORMALITY IN HUMAN CHRONIC MYELOID LEUKÆMIA

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THREE reports have been published which indicate that in some cases of acute leukæmia in man there are abnormalities of both number and morphology of the chromosomes in the leukæmic cells[1–3]. However, no consistent abnormality has yet been found which can be linked to a specific cytological type of acute leukæmia. Baikie *et al.*[1] also reported that they were unable to detect any chromosome abnormality in bone marrow preparations from a number of cases of chronic myeloid and chronic lymphatic leukæmia. Nevertheless, it was said at the time that the standard of the preparations was such that the possibility of a small chromosomal lesion could not be excluded.

In recent months considerable developments have occurred as a result of the utilization of the technique of Hungerford *et al.*[4] for the culture of the leucocyte component of the peripheral blood. It is now recognized that chromosome preparations made from such cultures are of an appreciably better quality than those made from bone marrow. Using this blood culture technique, Nowell and Hungerford[5] noted the presence of a minute chromosome in dividing cells from the peripheral blood of two male patients with chronic myeloid leukæmia, and they regarded this as an unusually small *Y* chromosome. At about the same time as these observations were published, we noted the presence, in blood culture preparations, of a similar small chromosome in two female patients with chronic myeloid leukæmia. It was apparent, therefore, that if the same abnormal chromosome was being viewed by both groups of workers, then the abnormality must involve an autosome and not the *Y* chromosome. In the light of these findings we re-examined all our material from cases of chronic myeloid leukæmia, as well as studying two fresh cases of the disease. As a result of this we are satisfied that the presence of an unusually small autosome (Fig. 1) is a characteristic feature of many cases of chronic myeloid leukæmia.

Altogether we have studied the chromosomes in blood cultures from 12 patients (4 males and 8 females) suffering from chronic myeloid leukæmia. Among these were two patients who had had no previous treatment for their disease, either by X-irradiation or by chemotherapy. The remaining 10 patients had all been treated by radiotherapy some time prior to our work. The small chromosome was noted in preparations from eight patients, inclusive of both the untreated patients. Included among the 12 patients were three in a terminal acute transformation of the disease, and the relevant chromosome was noted in cultures from one of these. Marrow preparations have been studied from 10 patients, of whom four were males, and the small chromosome has been clearly seen in three patients, all three being fresh untreated cases. These three latter patients include two of those in whom the chromosome was also seen in blood culture. The remaining seven marrow preparations were of too poor a quality for us to be

Fig. 1. Cell from the leucocyte component of blood cultured from a female with chronic myeloid leukæmia. The three normal-sized small acrocentric chromosomes are marked *A*. The unusually small acrocentric is marked *B*

certain as to whether the abnormality was present or not. In no case has the abnormally small chromosome been seen in every cell. The frequency of its occurrence has varied from 1/7 cells to 48/50 cells studied, and in all patients some normal euploid cells have been seen.

In addition to re-examining our material from cases of chronic myeloid leukæmia, we have also re-examined our preparations from the peripheral blood of individuals with apparently normal karyotypes, and our marrow and blood preparations from patients with acute leukæmia in which we had thought no chromosome abnormality to be present. Blood preparations were available from 90 individuals with apparently normal karyotypes and seven were selected at random, to which were added five preparations from cases of chronic myeloid leukæmia showing the small chromosome. These twelve preparations were then submitted to an independent observer who was ignorant of the patients involved. The five preparations with the small chromosome were readily identified, and in none of the seven control preparations was the chromosome thought to be present. We have also reviewed marrow preparations from eleven patients and blood preparations from six patients with acute leukæmia having an apparently normal karyotype, and we have no evidence in these of the unusually small chromosome. Finally, we have studied the blood cultures from five cases of mongolism associated with acute leukæmia and found no abnormality apart for the classical mongol karyotype which is presumed to be trisomy for autosome 21.

Before discussing the possible significance of these findings, the question of radiation exposure has to be considered, as chronic myeloid leukæmia is increased in frequency in heavily irradiated populations[6,7]. We have not been able to check with any certainty the histories of diagnostic X-ray exposure of our patients prior to the development of their leukæmic state. Two patients, however, have histories of radiotherapeutic exposure for conditions quite unrelated to leukæmia. One patient, a woman, was treated in December 1953, for a simple papilloma of the bladder by means of a radium implant. An area 3 × 3 cm. on the base of the bladder was given a dose of 6,000 rads over a period of 146 hr. This patient was diagnosed as having chronic myeloid leukæmia in May 1958, and the unusually small chromosome has been identified in cultured blood cells. The second patient, a male, was treated for ankylosing spondylitis between February and April 1955, a total dose of 1,500 rads being given to the skin over the whole length of the spine and the sacroiliac joints. A diagnosis of chronic myeloid leukæmia was made in March 1956. Only marrow cultures were available from this man and their quality was such that we could not be certain whether the small chromosome was present or not. On the basis of our knowledge of the distribution of the latent periods of radiation-induced leukæmias between radiation exposure and diagnosis, we cannot exclude the possibility that the first case was induced by radiation; it seems, however, unlikely that this was so in the second case, as the length of the latent period following a single heavy exposure usually lies between four and eight years, and the increased frequency of leukæmia is not noted until the second post-exposure year.

It now seems certain that the unusually small chromosome is one of the members of the two pairs of small acrocentric chromosomes numbered 21 and 22 in the Denver Classification. The most reasonable explanation for its small size is the occurrence of a deletion. The fact that we have in every preparation found some normal euploid cells suggests that the error does not occur in all the body cells and presumably is confined to cells from the bone marrow. We are at present undertaking the investigation of the chromosomes from skin culture preparations to help to clarify this point*. We have not noted the unusually small chromosome in any other person that we have studied. Carter *et al.*[8], however, have published ideograms of the chromosomes from blood cultures made from a mother and her mongol son, and in both these ideograms an unusually small chromosome appears to be present. The mother has a diploid number of 45, and is considered to have a translocation involving autosomes numbers 15 and 21, while the son has the full complement of small acrocentrics in addition to the same translocation, and is presumed to be effectively trisomic for number 21.

It has been established that mongols have a heightened probability of developing acute leukæmia[9–13], but not apparently of developing chronic myeloid leukæmia. From unpublished observations on death certificate data (Court-Brown and Doll) it does not appear that this increased liability relates to any specific cytological type of acute leukæmia, although the unreliability of such data must be borne in mind. It is also known that in most mongols there is a morphological abnormality of the polymorphonuclear leucocytes which takes the form of a deficiency in nuclear lobulation[14]. It is not unreasonable, therefore, to suggest that the autosome involved in mongolism may possess a locus or loci concerned with some aspect of the control of leucopoiesis. Our present findings in relation to chronic myeloid leukæmia indicate that in many cases of this disease there appears to be a deletion involving either autosome 21 or 22. In the meantime it would seem not unreasonable to adopt the working hypothesis that the same autosome is involved in many cases of chronic myeloid leukæmia and in mongolism.

**Note added in proof.* Since this communication was submitted for publication, it has been possible to examine the chromosomes in cells from skin cultures of three patients with chronic myeloid leukæmia. Blood culture studies of all three patients showed the presence of the unusually small chromosome, but this was not seen in any of the skin cells.

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BLUE WILDEBEEST AND THE ÆTIOLOGICAL AGENT OF BOVINE MALIGNANT CATARRHAL FEVER

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MALIGNANT catarrhal fever of cattle is a transmissible disease of world-wide distribution, sporadic occurrence and very high mortality[1-3]; it is not contagious[1,2,4] and fomites are not infectious on parenteral inoculation[1]. The ætiological agent is generally held to be a virus although, to our knowledge, there has been no reliable proof of its filterability.

In the majority of countries it is believed, largely on circumstantial evidence, that sheep are reservoirs of the virus without ever showing any clinical signs of its presence[2,5-8]. However, the mode of transmission from sheep to cattle has not been determined, and, in fact, the full clinical and pathological sydrome has never been reproduced in cattle by inoculation of materials from sheep. In Africa, infections among cattle often follow close association with apparently normal wildebeest[1,7,9,10], but the only experimental proof that the latter may harbour the agent of malignant catarrhal fever was provided by Mettam[1]. In one instance he inoculated the blood of a black wildebeest (*Connochaetes gnou*, Zimmerman) into an ox which showed a transitory pyrexia 14 days later. Blood collected at this time caused typical malignant catarrhal fever on sub-inoculation to another ox. Further attempts to demonstrate the virus in wildebeest consistently failed.

We have recently investigated the role of the blue wildebeest (*Gorgon taurinus taurinus*, Burchell) in the epizootiology of malignant catarrhal fever in Kenya. The causal agent has been isolated from them and successfully propagated in cattle, rabbits and monolayer tissue cultures; the latter, especially, have thrown new light on its salient characteristics, which are here described in a preliminary manner.

During February 1959 and February–March 1960, 35 blue wildebeest of varying age and sex (Table 1) were shot in two game reserves of Kenya which are about 100 miles distant from each other. Heparinized blood and, occasionally, spleen and lymph-node tissue were collected immediately, stored on ice and within 48 hr. inoculated into cattle by the intravenous or subcutaneous routes. Monolayers were prepared from trypsinized cells derived from five near-term fœtuses and four calves 1–3 months old; all these cultures were observed for 28 days to detect cytopathic changes. Finally, leucocyte fractions from about 200 ml. of blood of 20 wildebeest were inoculated into monolayer cultures of normal bovine thyroid cells (*vide infra*) and suspensions of two fœtal spleens were used for cattle and culture inoculations.

Five isolates of malignant catarrhal fever virus were recovered from the 35 wildebeest, three from females in late pregnancy, one each from a yearling male and a yearling female (Table 1). Animal inoculations yielded three isolates, bovine thyroid cultures two different strains, one of which was shown to produce typical malignant catarrhal fever in cattle. No infective virus was demonstrated in lightly centrifuged suspensions of pooled spleen and lymph node tissue from the two pregnant animals which were proved to have had a viræmia.

Table 1. ISOLATION OF MALIGNANT CATARRHAL FEVER VIRUS FROM BLUE WILDEBEEST IN KENYA

Age	Sex	No. of animals	No. positive on cattle inoculation*	No. positive by all tissue culture tests	Remarks
Mature† Pregnant	F	8	3/8	0/8	One isolate recovered from fœtal spleen only
Mature	F	4	0/4	0/3	—
Yearling	F	6	0/6	1/6	First cytopathic changes, day 16
< 3 months	F	2	0/2	0/2	—
Mature†	M	7	0/7	0/5	
Yearling	M	6	0/6	1/3	First cytopathic changes, day 18
< 3 months	M	2	0/2	0/2	
Totals	—	—	3/35	2/29	5 isolates recovered from 35 wildebeest

* Blood was inoculated in a dose of 25 to 200 ml., tissue suspensions contained 3–10 gm. material.

† Mature means estimated to be two years or older.

The disease developed in cattle after an initial incubation period of 34–38 days, being clinically identical in all respects to the virulent 'head-and-eye' form[11]. Pathological and hæmatological changes in infected cattle were similar to those described previously for cattle-derived strains of this virus[10-12]. In addition to the well-known perivascular accumulations of mononuclear cells, there was an angiitis of both arteries and veins characterized by necrosis and mononuclear cell infiltration of the media with swelling and proliferation of the endothelium[12].

One isolate was carried by two methods through more than 20 serial passages in cattle. The first line was maintained by the intravenous inoculation of heparinized blood, 5 ml. being an effective dose; for the second, crude 10 per cent suspensions were prepared in Ten Broeck grinders from prescapular lymph-node tissue and 5 ml. quantities of these were inoculated subcutaneously. Once established in cattle the incubation period decreased to a mean $18{\cdot}5 \pm 1{\cdot}1$ days, and the method of sub-inoculation did not

produce significant variations in this parameter. The mortality was 96 per cent with an average survival time of 8·7 ± 0·8 days.

Ten per cent lymph-node suspensions of the third to twenty-fourth passages produced the disease in cattle after 10^{-2}–10^{-3} dilution. Nevertheless, the supernatants remaining after centrifugation for 10 min. at 2,000 r.p.m. were devoid of infectivity in a dose of 20–25 ml. Similarly, 100 ml. of plasma was non-infective although whole blood had a titre of 10^{-1}–10^{-2} per 5 ml. These observations confirmed and extended previous findings[13,14].

The agent was established in rabbits[10] by the intracerebral or intraperitoneal inoculation of cattle lymph-node tissue and passaged by the intracerebral inoculation of brain or the intraperitoneal injection of spleen or lymph-node suspensions. The average incubation period in this species was 15·2 ± 0·8 days, death following invariably and usually suddenly after a further 4·6 ± 1·3 days. The post-mortem findings and histopathology were similar to those already described[10,12]. Plasma or low-speed supernatants from suspensions of infected tissues sometimes contained infective virus, thus differing from cattle materials treated similarly.

Initial attempts to propagate the virus in tissue culture were made by preparing monolayers from the kidneys, bone marrow and blood leucocytes of infected cattle. No cytopathogenic effect was observed at this time in living or stained cultures during an observation period of 28–30 days. Lymph-node suspensions produced no specific changes in established primary cultures of calf or sheep embryonic kidney and calf or sheep testis. Stimulated by the work of Pulvertaft[15], we then prepared monolayers from trypsin-dispersed cells of the thyroid of an infected ox, and these cultures showed a focal cytopathogenic effect first observed on the ninth day. The causal agent could not be passaged with cell-free fluids from the infected culture, but subcultivation to other thyroid monolayers was achieved by intact cells removed from the glass mechanically or with a mixture of versene and trypsin. These cell suspensions also reproduced malignant catarrhal fever in cattle following intravenous administration. Stained coverslip preparations showed that the cytopathic changes observed were characterized by the formation of syncytia with intranuclear inclusions of type *A* (Fig. 1).

Similar cytopathogenic agents were regularly recovered by culture of the thyroid glands of other diseased cattle, infected with the same and other wildebeest isolates. The incubation period, when cell growth was satisfactory, was about 6–8 days. Furthermore, it was found that monolayers were readily prepared from adrenal gland tissue of infected cattle, and that these showed identical focal cytopathic changes, frequently detectable after 5–9 days. Virus of similar cytopathogenicity was also demonstrated in the blood of infected wildebeest (Table 1), cattle and rabbits and in lymph-node suspensions from the last two, by inoculation on to normal calf thyroid monolayers. Cytopathic changes were seen after as little as 3–4 days in the case of the last two species, blood titres reaching $10^{3\cdot3}$ TCD_{50} per ml. and gland titres $10^{4\cdot4}$ TCD_{50} per gm. in the ox. No cytopathogenic agent was ever recovered from uninoculated control cultures observed over prolonged periods.

Cell types which were found to be susceptible to the virus isolated in thyroid cultures included primary sheep thyroid, calf testis and adrenal or rabbit and wildebeest kidney. In all these, however, as also in bovine thyroid, there was a tendency for the virus-induced changes to become progressively less extensive on passage or prolonged cultivation. Very satisfactory serial cultivation was eventually achieved by employing an 'established' line of calf kidney cells developed in this laboratory by one of us (R. D. F.). Suspensions were mixed, at the time of seeding into culture vessels, with infected trypsin-dispersed cells of the previous passage. Cytopathic foci then appeared after as little as 24 hr., often extending to involve a large proportion of the available cells in 5–7 days.

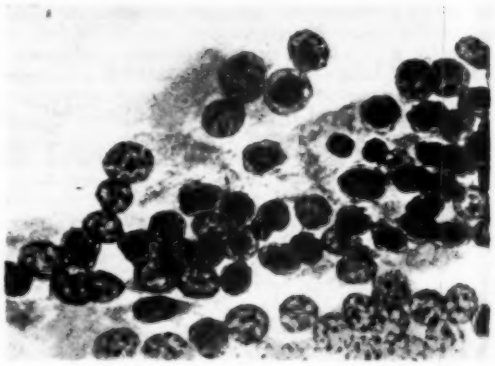

Fig. 1. Thyroid monolayer from an infected ox, eight days after seeding, showing a nuclear aggregation with intranuclear inclusions

Five isolates were passed 14–38 times in this manner, the total period of time in culture varying from 3 to 10 months. Full cattle pathogenicity was retained at least up to the tenth to nineteenth passages for three isolates tested. Trypsin-dispersed cells from infected cultures were titrated by mixing 10-fold dilutions in growth medium with 'clean' suspensions of the cell line. The maximum number of infective centres was approximately 10 per cent of the total viable units as estimated by single cell counts after staining with neutral red. A suspension containing $10^{4\cdot9}$ TCD_{50} per ml. of fourteenth culture passage virus had a cattle infective titre of $10^{3\cdot2}$.

For a variable period no virus could be demonstrated in cell-free fluids from infected cultures, but after seven to thirty passages it was found that all four isolates produced free virus. This was not deposited at 5,000*g* for 30 min. and reached a titre of $10^{2\cdot3}$ TCD_{50} per ml. in one instance. Free virus was shown to be capable of passing graded collodion membranes of average pore diameter 430 mμ.

The cause of malignant catarrhal fever is therefore a virus which is present in the tissues of a proportion of apparently normal wildebeest in two widely separated areas of Kenya. The recovery of the virus from the spleen of a wildebeest fœtus *in utero* (Table 1) showed that trans-placental transmission occurs in this species. It can be propagated in suitable monolayer tissue cultures and produces a cytopathology which resembles that of herpes simplex[16], *B* virus[17], pseudorabies[18], varicella and herpes zoster[19]. It is remarkable that the histopathology of malignant catarrhal fever in cattle and rabbits has never been

reported to include either the formation of syncytia or the presence of intranuclear inclusions. A careful re-examination of material available to one of us (W. P.) has confirmed the absence of both these features.

Epizootiologically malignant catarrhal fever virus behaves like *B* virus, herpes simplex and pseudorabies in that these agents usually cause very mild infections in their customary reservoirs (monkey, man and pig, respectively) but highly fatal diseases when introduced into other experimental or natural hosts[20,21]. Herpes is a classic example of persistent infection, and some authorities[22,23] consider that herpes zoster may be a manifestation of recurrent pathogenic activity in individuals harbouring the virus of varicella. A viræmia extending over many months has been proved in this laboratory to be present in some of the few cattle which recover from malignant catarrhal fever. It is probable that the same virus persists for long periods, if not for life, in sheep and wildebeest.

The agent of malignant catarrhal fever resembles varicella and herpes zoster in its failure to produce free virus in early culture passages[19], but differs from them in that all tested isolates do so later and that cell-free virus cannot be demonstrated in the tissues of infected cattle. This last is a most important fact, which probably explains many failures to transmit the disease experimentally and also to filter and conserve the causal agent. In connexion with the last problem it may be noted that methods employed for the preservation of cell lines at low temperatures have been shown by us to be suitable for the long-term storage of the agent in cultured cells. The absence of free virus in cattle has an obvious bearing on the technique and interpretation of quantitative studies on the multiplication and distribution of the virus in tissues of infected animals. It is also fundamental to a study of its resistance to various physical and chemical agents, or neutralization by 'immune' sera.

The peculiar sensitivity of thyroid and, to a less extent, adrenal cells for the primary isolation of malignant catarrhal fever virus and the failure of other more widely employed types suggests that the former may possibly be used with advantage for other viruses which have so far failed to produce cytopathogenic effects *in vitro*.

More detailed results of these and other investigations will be published elsewhere.

We are grateful to Capt. D. N. Zaphiro, Kenya Game Department, and to Lee M. Talbot, Wildlife Research Project, U.S. National Academy of Sciences, for invaluable help in the collection of tissues from wildebeest.

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ROLE OF CEREAL FAT IN THE PRODUCTION OF NUTRITIONAL DISEASE IN PIGS

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IN Sweden nutritional diseases such as hepatosis diaetetica (dietetic liver necrosis) and muscular degeneration represent a serious hazard in pig raising. Through pathological and experimental work, Obel[1] demonstrated that two factors—vitamin E and the sulphur-containing amino-acids, methionine and cystine—are effective in preventing hepatosis diaetetica and suggested that Schwarz's factor 3 might also be involved. Since Schwarz and Foltz[2] detected the factor 3 effect of selenium compounds, and Eggert *et al.*[3] and Grant and Thafvelin[4] have shown the effectiveness of selenium salts for the prevention of experimental hepatosis in pigs, and since Schwarz *et al.*[5] have established that the protective effect of the sulphur-containing amino-acids almost certainly depends on contamination with selenium, attention is now mainly directed towards vitamin E and factor 3 (that is, biologically active selenium) in the ætiology of the lesions concerned. The absolute vitamin E content of feeds is *per se* of interest; but it is the relationship between the amounts of vitamin E and polyunsaturated fatty acids which seems to deserve emphasis. For this reason the unsaturated fatty acids of cereal grains have assumed a central place in current work on hepatosis diaetetica and muscular dystrophy in pigs.

In spite of conventional prophylaxis through dietary manipulation (cod liver oil is nowadays fed only sparingly to pigs), too many cases of hepatosis diaetetica and muscular degeneration occur among pigs in Sweden. There is reason to look into the ætiological importance of the grain fed since these diseases often occur among pigs fed nearly exclusively on grain.

Table 1. Properties of the Various Feeds

Feed	Fat content (per cent)	Iodine No.	Free fatty acids ml. KOH/gm. fat	Peroxide value m.equiv./kgm. fat	Tocopherol content (α-tocopherol) mgm./100 gm.
Feed I, unheated	4·8	78	77	0	2·36
Feed I, heated	3·7	37	58	140*	0·48
Feed II, unheated	3·8	75	135	164	0·40
Feed II, heated	2·7	76	130	0	0·67
Maize oil, fresh	—	112	1·2	0	69·0
Maize oil, heated	—	109	1·4	250*	6·31

* Mean throughout experiment.

Theoretically, there are two aspects of the disease-producing properties of feed grains to be considered—the unsaturated fatty acids and the level of biologically active selenium. In a recent paper[6], we have published the results of work on cereal fats and have indicated their potentialities for becoming rancid in certain circumstances. This article gives a brief account of attempts to produce nutritional lesions in pigs by feeding grain containing rancid fats or fats with a tendency towards autoxidation. The results were checked by feeding pure vegetable oil of known oxidative status.

Apart from the experimental feeds to be described here, the pigs received daily supplements of 1–1·5 litres skimmed milk and minerals. Pigs of the Swedish Lantras breed, 10–12 weeks old, were used. Routine blood analyses and determinations of serum transaminase activities (glutamic-oxalacetic transaminase (GOT) according to Karmen-Ordell[7]) were performed. Our normal value for serum-GOT activity in pigs is $22 \cdot 5 \pm 7 \cdot 7$ ($n = 61$) Karmen–Ordell units. Through work on pigs of the same breed carried out by Reitman and Frankel's method, a normal value for serum-GOT activity of $28 \cdot 03 \pm 15 \cdot 39$ units was found by Wretlind, Orstadius and Lindberg[8].

'Peroxide'-levels in the cereal fat and vegetable oil were estimated by a method developed by K. Erne of this Institute.

The tocopherol contents were determined in the laboratories of AB Ewos, Södertälje, by Dr. G. Ekström using the method of F. Bro-Rasmussen and W. Hjarde[9]. All pigs which died or were killed were autopsied (C. A. Grant[4]). The lesions of hepatosis dietetica and muscular degeneration were evaluated by conventional standards[1]. The criteria applied for the diagnosis 'plötzlicher Herztod' have been briefly mentioned before[4] and will be presented more fully in another connexion.

Experiment 1. The effect of feeding grain containing fats of great susceptibility towards rancidity (feed I).

As shown in Table 1, autoxidation could be induced in this sample of grain by heating in an incubator (21 hr. at 100° C.). Five pigs received grain heated in this manner and five received unheated grain from the same sample. Significantly increased serum-GOT values were recorded for the first group from the twenty-first day (Fig. 1). Two died after 21 and 33 days, respectively, and the remaining three were killed after 56 days. The major autopsy findings are given in Table 2.

The five control pigs remained clinically healthy. All were killed on the thirty-sixth day. From the transaminase values given in Fig. 2 and from experience with previous experiments it appears most likely that tissue destruction did not occur during the experimental period. On several occasions during the last days of the experiment the pigs fought savagely; this experiment is to be repeated with precautions to eliminate fighting.

Experiment 2. The effect of feeding grain containing fat which had spontaneously oxidized during storage (feed II).

This sample of grain had produced nutritional lesions among pigs on a farm. Five pigs were given the feed as such. Five control animals received the grain heated in an incubator at 100° C. for 4 hr. at the beginning of the experiment and later for 6 hr. to accelerate the oxidative processes to stability (Table 1). Increased serum-GOT values were seen in the pigs of the first group by the sixth day (Fig. 3). One pig died on the thirteenth day, two on the fourteenth, and the remaining two were killed on the thirty-eighth and fortieth days, respectively.

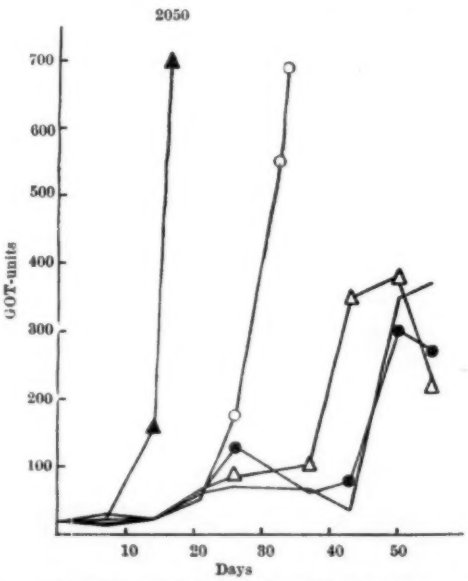

Fig. 1. Serum enzyme-levels for pigs fed grain heated in order to induce oxidation

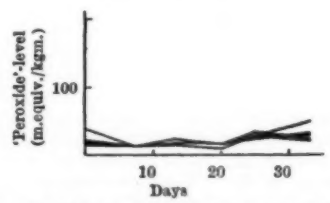

Fig. 2. Serum enzyme-levels for pigs fed unheated grain

Table 2. Major Autopsy Findings

Group	No. of animals	Lesions of 'plötzlicher Herztod'	Waxy degeneration of skeletal muscles	Hepatosis diaetetica
Feed I, unheated†	5			
Feed I, heated	5	4	5 +++*	0
Feed II, unheated	5	5	5 +++	1
Feed II, heated	5	0	5 + to ++	0
Maize oil, fresh	2	0	1 (+)	0
Maize oil, heated	2	2	2 +++	1

Degree of degeneration:
*(+), only a few fibres involved; +, slight degeneration; ++, moderate degeneration; +++, pronounced degeneration.
† See text under experiment 1.

No clinical signs were seen in the five control pigs although all showed slightly increased serum-GOT activities (Fig. 4).

The major autopsy findings are given in Table 2.

Experiment 3. The effects of feeding fresh and rancid vegetable oil.

As a pilot experiment four pigs were fed a normal diet to which was added a daily supplement of 250 ml. maize oil per pig. Two pigs received fresh oil and two oil which had been oxidized by heating (Table 1).

The pigs fed rancid maize oil died after 16 and 37 days respectively. Serum-GOT values were elevated throughout the course of the experiment (Fig. 5).

No clinical signs and no increase in serum-GOT activities were evident for the two control animals (Fig. 6).

The major autopsy findings are listed in Table 2.

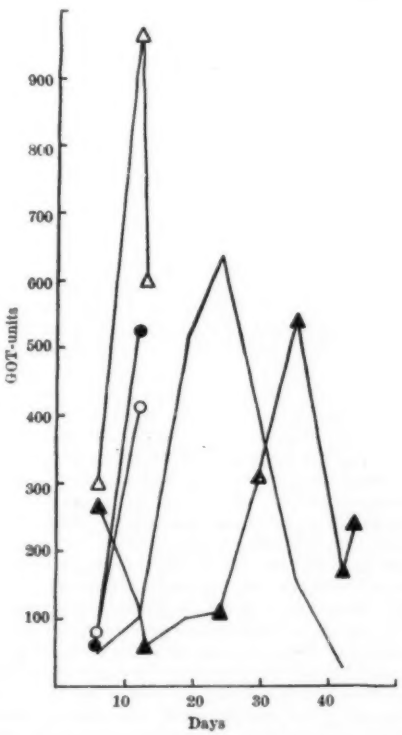

Fig. 3. Serum enzyme-levels for pigs fed grain containing fat which had spontaneously oxidized during storage

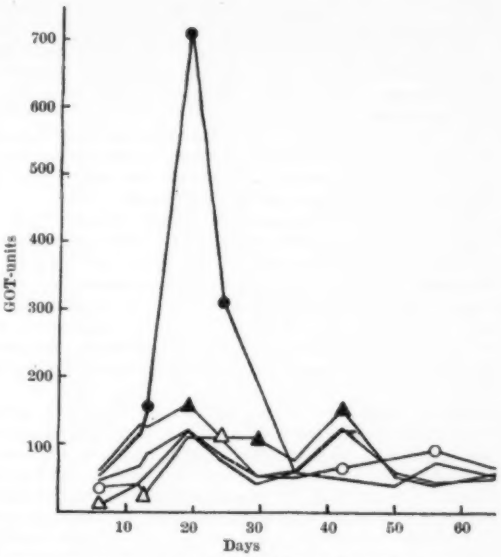

Fig. 4. Serum enzyme-levels for pigs fed grain heated in order to accelerate the oxidative processes to stability

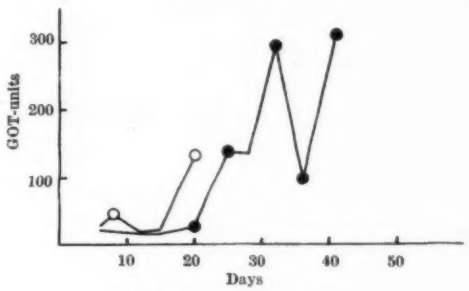

Fig. 5. Heated maize oil

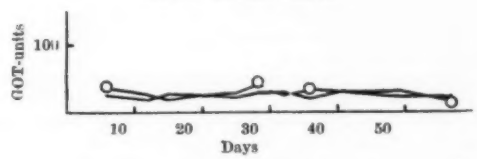

Fig. 6. Fresh maize oil

Discussion

It has long been known that hepatosis diaetetica, muscular dystrophy and allied lesions can be produced experimentally by feeds which contain unsaturated fatty acids of marine origin[1,10–14]. It is, however, an observed fact that the addition of marine fat to pig feed is by no means a prerequisite for the occurrence of these lesions in Swedish pigs. Grain, on the other hand, forms the greater part of conventional pig rations. With this in mind, the question could be posed whether or not the natural fat of the grain could in certain circumstances be the underlying cause of these disorders. Chemical studies of the properties of cereal fat during various phases of maturation and under various storage conditions have shown this hypothesis to be theoretically accept-

able[6,15–17]. The results of this initial work can be summarized as follows.

(1) 'New grain' contains fats characterized by little or no rancidity but also by great susceptibility to rancidity. This susceptibility steadily decreases during storage.

(2) If ground, grain will gradually lose the ability to protect its fat against oxidation and, depending on its original properties, sooner or later during storage in this state will become rancid.

Expressed in another way, grain can in certain circumstances contain unstable fats, that is, fats with a tendency to become rancid. This is especially apparent during rainy years with little sunshine. In experiment 1, grain of this type was ground and heated to give it roughly the same properties as ground grain stored under natural conditions. Treated in this way, the grain produced the lesions listed in Table 2.

The grain fed in experiment 2 was known to have produced spontaneous disease in a herd of pigs. The grain as such contained highly oxidized fats and produced the lesions described in Table 2. Through heating, it was possible to stabilize the fat and in that way greatly reduce its disease-producing propensity.

Finally, it could be shown in experiment 3 that pure vegetable oil, when oxidized, can produce lesions belonging to the same group as those encountered in the other two experiments and in pigs dying of the spontaneous diseases.

To sum up, then, there is ample evidence suggesting that when hepatosis diaetetica, muscular degeneration and allied disorders are seen in pigs fed mainly on grain, the occurrence of these lesions can be coupled with the properties of the cereal fat.

These investigations have been supported by grants from Jordbrukets forskningsråd. GOT reagents have kindly been supplied by AB Kabi, Stockholm.

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THE BIOSPECIES IN ENTOMOLOGY

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AT a recent joint discussion of the Linnean Society and the Systematics Association on the subject of the sub-species, it was quite evident that there is still no basic agreement between biologists on the nature of the species itself. Thus there can be little hope of defining the sub-species to the satisfaction of all workers.

It has been argued recently[1,2] that the term species should be restricted entirely to a morphological entity: that is, it would be the lowest taxonomic category (except the variety ?) above the individual and be defined entirely morphologically. In considering sexually reproducing forms, the justification for such a concept is that it represents the nearest approach to a true biological species which can be attained in the present state of knowledge for many animal and plant groups. Even to Linnæus the species was a biological entity characterized by a quality of breeding true[3]. Later workers stressed the importance of a sterility barrier between species. Poulton[4] developed the idea of the species as a freely interbreeding series of populations in Nature and coined the term 'syngamy' for such a free-interbreeding relationship. Even at the beginning of this century Poulton[4] was aware of the difficulty of determining the status of geographically isolated or allopatric forms, since it was not possible to determine whether or not such forms could interbreed in the field. This led to the modern polytypic species taking account of variation in space, that is, geographical variation. The concept has been fully developed by Mayr[5] and critically reviewed by Cain[6]. Unfortunately, the status of truly allopatric forms can never be determined with certainty. However, sympatric forms, that is those the breeding ranges of which either coincide or overlap in Nature, can be delimited objectively by the presence or absence of syngamy, of what Mayr[5] has termed "crossability", between them. There is no biological reason why morphological differences should always be associated with the breeding barrier and thus no *a priori* reason why there should always be morphological differences between species. Of course there usually will be such differences which can then be used as indicators of the true biospecific limits. This is the main justification for the use of the morphospecies in neontology when considering bisexually reproducing forms.

Dobzhansky[7,8] has developed the theory of speciation by spatial isolation of populations and subsequent selection for isolating mechanisms. It is supposed that the populations would diverge genetically when in spatial isolation due to the differential action of natural selection in the different environments. On meeting again, hybrids between the forms would be at a selective disadvantage to either of the parental types, and, providing that this disadvantage was large enough not to be swamped, there would be heavy selection for the establishment of isolating mechanisms and eventually separation as good species. Koopman[9] has been able to demonstrate the very

rapid evolution of isolating mechanisms in the laboratory between two very closely allied species of *Drosophila*, *D. pseudoobscura* Frolova and *D. persimilis* Dobzhansky and Epling, which can be made to interbreed at temperatures of about 16° C. These experiments strongly support Dobzhansky's theory, which also seems adequate to explain the existence of many groups of insects in which morphological specific differences are either very small or almost non-existent. In such groups the species often differ more obviously in biological features.

It is essential that there should be some ecological differences between closely allied sympatric species if they are to co-exist. Ecological differences such as separate breeding seasons may have arisen when the populations were spatially isolated and would then automatically be important as isolating mechanisms when the populations met again. However, some ecological differences may not be of importance in the actual process of speciation, but only in the avoidance of competition after specific differentiation. Thus differences between closely allied sympatric species may be of at least two kinds, associated either with the primary isolating mechanisms or with secondary ecological differences and usually with both. However, morphological characteristics need not always be associated with either. Thus in my view it is wrong to insist on distinct morphological characters for separating species when the only certain criterion is the presence of reproductive isolation. Perhaps the most satisfactory definition of the biological species or biospecies is that of Mayr[5], namely, "Species are groups of actually or potentially interbreeding natural populations, which are reproductively isolated from other such groups". The only weakness lies in the phrase "potentially interbreeding", which has to be introduced in order to include allopatric populations.

All grades of morphological differences between true biospecies have been recorded, including many in which the differences are very slight. Well-known examples of such sibling species are *Drosophila pseudoobscura* and *D. persimilis*[10] and the species of the *Anopheles maculipennis* group[11]. Undoubtedly many further examples exist and will be uncovered during the course of intensive biological investigations which are now being carried out in many insect groups. Recently, Claridge and Askew[12] have described a group of siblings in the genus *Eurytoma* (Hym., Chalcidoidea). *E. rosae* Nees and *E. brunniventris* Ratz. are inseparable on the adult characters which I have been able to study, but they differ in characters of the egg and in details of hosts and life-histories. Also in the family Eurytomidae, Neunzig and Gyrisco[13] have recently shown that *Bruchophagus platpyterus* (Walk.) (= *gibbus* Boh.) is an aggregate of at least three species with definite host plant preferences. There is also evidence of the widespread occurrence of sibling species in other groups of Chalcidoidea.

The desire to retain the species as a static morphological entity with little reference to biological reality must be overcome if taxonomy is to make further contributions to biological science. It may be, as suggested by Gilmour[2], that the problems in botanical taxonomy are so great that a completely new system of categories is required. However, this would be a very unfortunate change since it would obscure the similarities between the real biological entities of both zoological and botanical taxonomy.

I thank Dr. A. J. Cain for discussion on the subject-matter of this article.

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SOME OXIDATIVE AND HYDROXYLATIVE ACTIONS OF QUARTZ: THEIR POSSIBLE RELATIONSHIP TO THE DEVELOPMENT OF SILICOSIS

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THE existence of a special surface activity possessed by siliceous dusts and silicates has been frequently reported in the literature. Hauser[1] indicated that the formation of a free silica surface was accompanied by the liberation of protons and the formation of atomic oxygen, and found a correlation between particle size and amount of oxygen released. In essence, he postulated that the unusual oxidation potential of silica resulted from the decomposition of some superoxide ions to yield atomic oxygen. Weyl[2] considered a type of dynamic hydration–dehydration equilibrium which would account for the fact that siliceous materials were able to act as oxidation catalysts in the presence of air.

Our interest was given stimulus when we found that quartz dust could be employed successfully as an oxidant in a number of *in vitro* oxidation reactions, and we have since verified some of the postulates set out above.

Since the conversion of proline to hydroxyproline can be readily effected by hydrogen peroxide, we have been able to estimate quantitatively the oxidative activity of a number of dusts of known particle size as 'peroxide equivalents' per unit weight per

unit time for this conversion. The peroxide equivalent of a dust is obtained by comparing the amount of hydroxyproline (colorimetrically at 560 mμ) which can be produced by 1 gm. of dust shaken with 5 mgm. of proline for 30 min., with that amount produced by 2·5 mgm. of hydrogen peroxide under the same conditions. Table 1 shows a list of dusts in decreasing order of activity; in addition to the dusts listed, 'Aerosil' (250–500 Å. 'colloidal silica') has since been shown qualitatively to hydroxylate proline.

On the face value a correlation appears to exist between the 'oxidative activity' of the dusts and the known fibrogenetic potential of the materials examined. Crocidolite, however, is an exception; there are a number of suggestions for its apparently anomalous behaviour and these are being investigated together with the behaviour of other silicates. A further connexion between particle size and activity also seems apparent in the case of coarse quartz and the other quartz dusts of a much smaller size. Whether there is any relationship between oxidation and surface area is at present being examined.

It has been shown in the laboratory (Table 2) that quartz can bring about a number of oxidative and hydroxylative conversions, many of which have been previously demonstrated in biological systems. In all cases the products were identified by paper chromatography with specific detection reagents and by the use of pure standards, and wherever possible the identity of the products was checked by colorimetric estimation, followed by examination of the absorption spectra obtained.

Particularly interesting conversions which have been listed above are the hydroxylations of proline and lysine to yield small amounts of hydroxyproline and hydroxylysine, respectively, amino-acids found almost specifically in collagenous protein. In addition, glycine, another important amino-acid of collagen, has been shown to be produced by quartz.

The maximum yield of hydroxyproline obtained with quartz under optimum conditions was 0·1 and 0·16 per cent in the presence of vitamin C. In most cases the yield was improved by dry shaking instead of wet, and in the case of hydroxyproline, it was enhanced by the addition of certain co-factors, the most important of which was ascorbic acid; this increased the yield up to 60 per cent. The additional observation was made that ascorbic acid can feebly hydroxylate proline *in vitro*, and this raises the question whether quartz does not actually synergize the ascorbic acid hydroxylation of proline.

Table 1. Oxidative Activity of Various Dust Samples in Terms of their Ability to Convert Proline to Hydroxyproline

Sample*	Per cent size distribution (μ)	Peroxide equivalent (mgm.)
Superfine quartz dust	87 < 1	3·25
Acid-washed quartz dust	92 < 1	3·00
Mine dust (Witwatersrand gold mine)	84 < 1	2·70
Coarse quartz dust	90 > 5	1·75
Coal dust	86 < 1	1·00
Crocidolite	86 < 1	nil
Kaolin	92 < 1	nil
Talc	76 < 1	nil

* The materials used have the following characteristics: superfine quartz dust (0·03 mgm. iron/gm.) (Dowson and Dobson); acid-washed quartz (trace of iron present after washing with hydrochloric acid); mine dust (54 per cent silica) (Witwatersrand gold mine); coarse quartz dust (0·05 mgm. iron/gm.), prepared by sedimentation; coal dust: anthracite (6·6 per cent silica); crocidolite: blue asbestos (50 per cent silica, 40·5 per cent ferrous oxide and ferric oxide); kaolin, washed and ignited, technical (J. T. Baker Chemical Co., Phillipsburg, N.J.); talc: hydrated magnesium silicate.

Table 2. Oxidative and Hydroxylative Conversions produced by Quartz after Vigorous Shaking with Starting Materials

Material	Product	Other oxidants used for confirmation
dl-Proline	Hydroxyproline*†	H_2O_2
β-Alanine	Glycine†	H_2O_2 + Fe salts
Lysine	Hydroxylysine†	H_2O_2 + Fe salts
Phenylalanine	Tyrosine*	H_2O_2
Ascorbic acid	Dehydroscorbic acid*	Alumina
Thiamine	Thiochrome†	$K_3Fe(CN)_6$
Adrenaline	Adrenochrome†	H_2O_2; $K_3Fe(CN)_6$
N-N dimethyl *p*-phenylenediamine	Würster's red†	H_2O_2
p-Phenylenediamine + α-naphthol	Indophenol purple†	H_2O_2
Calcium gluconate	Arabinose*†	Fenton's reagent (H_2O_2 + Fe salts)

* Dry shaking for 1 hr., then taken up in distilled water.
† Wet shaking throughout.

Hydrogen peroxide gave a greater yield of hydroxyproline compared with that given by quartz, and quartz added to a standard hydrogen peroxide–proline system gave the highest amount of hydroxyproline obtained. It is clear from Tables 1 and 2 that parallels exist between hydrogen peroxide and quartz-sponsored oxidations, and this suggests that quartz is functioning in a peroxidative capacity in most of the cases investigated. Kalyankar *et al.*[3] obtained similar conversions with peroxide in the presence of iron salts.

In general, it seems evident that the conversions sponsored by quartz are catalytic ones in the presence of air, and possibly of the type mentioned by Johnson *et al.*[4], where reactions involving free radicals *in vitro* were shown to simulate certain enzymatic processes.

It is not at the moment possible to relate these findings directly to the development of silicosis, and tentative suggestions only can be made. Quartz dust might give rise to peroxide in the tissues, or vitamin and hormonal activity might be interfered with at the site of deposition of dust. Furthermore, the possibility of the formation of toxic products from normal body constituents by the action of quartz should not be excluded: recent work (unpublished) in this laboratory by Marasas has shown that histamine and other imidazole derivatives are formed when histidine is allowed to react with quartz under simple laboratory conditions. In a different context, it has also been shown that measurable amounts of formaldehyde in some form or another can be readily detected in quartz and certain other dusts.

Finally, there is the possibility that quartz might in some way be involved in the direct synthesis of collagen from hydroxylated or oxidized precursor amino-acids or peptides, which might then be adsorbed on to the quartz surface in a manner similar to the template model proposed for such materials by Seifert[5].

We wish to thank the director of this Unit, Dr. H. S. Gear, for facilities extended to us during this work, the sub-director, Dr. Ian Webster, for helpful discussions, and Miss M. J. Matthews for undertaking particle sizing and counting.

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LETTERS TO THE EDITORS

ASTRONOMY

Bode's Law and the Solar System

In a recent communication[1] a model was proposed for the formation of the solar system. It was suggested that tides produced on the Sun by a passing star caused portions of the solar material to break away at regular intervals of time, the intervals being governed by the natural frequency of oscillation of the disturbed Sun.

The most outstanding feature of the solar system is Bode's law, which relates mathematically the radii of the orbits of all the planets other than Neptune. In fact, the form of the law

$$r \propto 4 + 3 \times 2^n \; (n = 0, 1 \ldots 7)$$

does not really include Mercury as a natural member. However, it is clear that the correct explanation of the origin of the solar system must include Bode's law as one of its consequences. It will be shown that a model of the type already proposed, with some modifications suggested by computational experience, explains the radii of the planetary orbits with reasonable accuracy.

The details of the process will be best understood by reference to Fig. 1. The Sun is considered to have been at rest (for illustrative purposes only—any mathematical analysis must take account of its motion about the centre of gravity of the system) and the star to have approached on an orbit which took it to within a distance, D, of the Sun. The solar tide grew as the star approached and rotated to face the oncoming star. Eventually, a point of instability was reached[2], and the tip of the tide broke away and moved henceforth under the combined gravitational influence of the Sun and star. It has been confirmed by computation that the embryonic planet would be very little affected in its subsequent motion by the star, and its orbit around the Sun may be calculated from the height, R, and the angular velocity, ω, of the tide at the time of its inception. This orbit, assumed elliptical, will have a major axis of length:

$$a = \frac{GMR}{2GM - R^2\omega^2} \text{ and eccentricity } e = \left| 1 - \frac{R^2\omega^2}{GM} \right|$$

where M is the mass of the Sun and G the gravitational constant.

After an interval, t, another planet was formed. The value of R would have been the same as before, but ω would have been greater due to the angular acceleration of the star. The point of release of the planet may be defined by the angle θ shown in Fig. 1; it is assumed that ω was the angular velocity of the star at the appropriate value of θ. In fact, ω would be somewhat less than this value due to an inertial lag of the tide which would be expected to build up gradually as the star accelerated towards the perihelion of the orbit. Once this lag was sufficiently great, the process of detaching material from the Sun would have ceased.

The eccentricity of the original orbits will have been reduced by the passage of the planet through the material surrounding the Sun: tenuous though this may be, it has an appreciable effect over long periods of time. The planets for which $\omega < \sqrt{\frac{GM}{R^3}}$ have R as aphelion distance; with $\omega > \sqrt{\frac{GM}{R^3}}$, then R is the perihelion distance. As a general rule, the eccentricity should be smallest for the innermost planets. Mercury is a notable exception to this rule; another exception is Neptune, the orbit of which is almost circular. This provides the clue to the value of R, because the orbit of Pluto, which is rather eccentric, actually intersects that of Neptune. This suggests that the orbit of Neptune was originally almost circular and that R was approximately equal to the radius of that orbit.

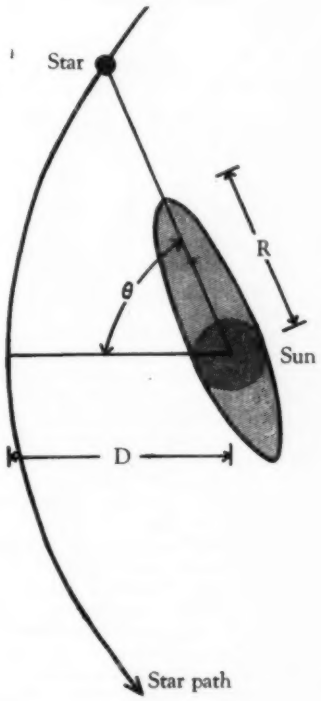

Fig. 1

Since the retarding medium has a density which falls off fairly sharply with increasing distance from the Sun, the characteristic of the orbit which should remain approximately constant is the perihelion distance, $a(1 - e)$, which depends on the value of ω. For the two outermost planets, which have been under no appreciable restraint, the function $a(1 + e)$ is that which corresponds to ω.

The values which are being considered at present are:

Perihelion distance of Sun–star orbit	$D = 5{\cdot}21 \times 10^{14}$ cm.
Tidal radius	$R = 4{\cdot}30 \times 10^{14}$ cm.
Mass of Sun	$R = 2{\cdot}20 \times 10^{33}$ gm. (about 10 per cent greater than at present)
Mass of star	$R = 3{\cdot}39 \times 10^{32}$ gm.
Period between planetary formation	$t = 2{\cdot}13 \times 10^{8}$ sec.

The star orbit is taken as parabolic.

The agreement between theory and observation is shown in Table 1.

Table 1

Planet	t ($\times 10^8$ sec.)	θ (°)	$a(1 \pm e)$ Theoretical ($\times 10^{12}$ cm.)	Observed ($\times 10^{12}$ cm.)
Mercury	?	?	?	0·46
Venus	0	96·4	1·09	1·08
Earth	2·13	92·3	1·53	1·48
Mars	4·26	87·5	2·12	2·05
X	6·39	81·8	3·17	—
Y	8·52	74·9	4·86	—
Jupiter	10·65	66·5	7·85	7·40
Saturn	12·78	56·1	13·42	13·43
Uranus	14·91	43·2	24·35	27·33
Neptune	17·04	27·5	45·00	45·30
Pluto	19·17	9·3	72·90	73·80

It will be seen that the agreement is generally good and that the following important features emerge: (i) Mercury does not fit into the system as, indeed, it does not fit properly into Bode's law. In view of the large eccentricity of its orbit, it is very unlikely to have started in its present course at the same time as the other planets. (ii) The model predicts two planets, *X* and *Y* in Table 1, between Mars and Jupiter. The asteroid belt may be explained as the debris of the collision of these planets. (iii) Neptune fits naturally into the planetary system and is not an odd member as Bode's law would suggest.

There have been substantial modifications of the model as originally put forward. In particular, the time-scale has been greatly increased (by a factor of about 10^5) and the origin of the asteroids is now explained as the results of a collision between two planets. However, the basic idea, that the planets were produced individually at regular intervals of time, has been preserved.

The full implications of the model are still being investigated, and, in particular, departures of the star-orbit from parabolic are being tried. Thus far, the model accounts satisfactorily for all those features of the solar system which have been examined.

The computations for this work were carried out on the University of Manchester *Mercury* computer, and I am indebted to the Electrical Engineering Department for the use of its facilities.

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Spheroidal Oscillations of the Moon

Periods of natural vibration of three spherical gravitating models of the Moon have been calculated on an IBM 7090 electronic computer. The values provide basic information for lunar space-research concerned with the design of experiments to investigate the structure of the Moon.

Three completely solid models of the Moon have been treated, each with radius, $R = 1{,}738$ km., and mean density, $\bar{\sigma} = 3{\cdot}33$ gm./c.c. Model I is a homogeneous sphere having throughout velocities for *P* and *S* waves of $\alpha = 8{\cdot}2$ km./sec., $\beta = 4{\cdot}7$ km./sec., respectively. Since only the differences between the principal moments of inertia of the Moon are known[1], there is no direct evidence, at present, that it does not possess a core or have a concentration of mass towards the centre. Thus the following Roche laws of density distribution which fit the total mass were used to define the other models:

Model II $\quad \rho = 3{\cdot}415 - 0{\cdot}135x^2$, $x = r/R$
Model III $\quad \rho = 4{\cdot}430 - 1{\cdot}830x^2$

The density distribution in Model II is close to that calculated by Jeffreys[2], for the self-compression of a Moon composed of ultrabasic rock with a surface density near 3·28 gm./c.c. Model III has a rather extreme density distribution having a surface value of 2·60 gm./c.c., near that of granite. This model was constructed to indicate the effect on the free periods of an internal lunar structure which has an increase of heavy material with depth (or a heavy core). Variations of *P* and *S* velocities with depth have been calculated in both Models II and III from the following velocity–density relationships which were based mainly on the *P* and *S* velocity distributions of Jeffreys[2] for the Earth and the density values of Earth Model A, constructed by Bullen[4]:

$$\left.\begin{aligned} \alpha &= -2{\cdot}40 + 3{\cdot}12\,\rho \\ \beta &= -0{\cdot}60 + 1{\cdot}52\,\rho \end{aligned}\right\} 2{\cdot}5 < \rho < 4{\cdot}5$$

(Linear velocity–density relations for longitudinal waves through considerable portions of the Earth's interior have been noticed by Francis Birch and J. E. Nafe (personal communication).)

The frequencies of the spheroidal oscillations were determined numerically from a sixth-order differential equation formulated by Hoskins[5] and Alterman *et al.*[6]. For this type of vibration the displacement has, in general, both transverse and radial components which are affected by the gravitational attraction. Integration was by a fourth order Runge–Kutta method, through homogeneous shells of thickness 100 km., using floating-point arithmetic and about eight decimal digits. Some solutions were also computed using a shell thickness of 50 km. and the agreement between corresponding frequencies was between 1 and 5 per cent. (For the present purpose it did not appear worth while to use additional machine time in order to improve the fit between calculations for a layered sphere and for a sphere with continuous properties by increasing the number of layers.) As a programme test, the period of oscillation of order 2 for a homogeneous Earth (called by Alterman *et al.* (*loc. cit.*) Model β) was computed, using shells 100 km. thick. The present programme yielded a period of 44·37 min. compared with 44·3 min. given by Alterman *et al.* The calculated lunar periods are shown in Table 1.

The case of the purely radial vibration ($n = 0$) of Model I allows a check on numerical accuracy. Pekeris and Jarosch[7] show that for a homogeneous sphere:

$$\varkappa R/\tan \varkappa R = (1 - \varkappa^2 R^2 \alpha^2/4\beta^2)$$

and

$$\varkappa^2 = (4\pi^2/T^2 + 4A)/\alpha^2$$

Table 1. Periods of Free Vibrations (min.)

n		Models I	II	III
0	Fundamental	8·73	8·7	7·5
	First higher mode	6·8	—	—
2	Fundamental	14·6	15·1	12·5
	First higher mode	8·0	8·4	8·1
	Second higher mode	4·9	—	—
3	Fundamental	9·8	10·2	8·8
	First higher mode	6·0	6·3	6·8
4	Fundamental	7·7	8·0	7·2
	First higher mode	4·8	5·1	5·6
6	Fundamental	5·6	5·8	5·5
	First higher mode	3·6	—	—

where T is the vibration period, and surface gravity is AR. For Model I the highest solution of these equations is $T = 8{\cdot}75$ min., in close agreement with the corresponding value in Table 1.

Concentration of mass towards the centre causes a relatively large reduction in the fundamental periods for $n < 6$. It is probable that for $n > 6$ the free periods of Models II and III again become markedly different (in the opposite sense) because motion would be largely confined to the surface region. The above results indicate that a measure of the deviation of the Moon from homogeneity is possible using observations of the long-period spectrum of free vibrations. An investigation of Press *et al.*[8] has shown the capabilities of a single short-period seismometer in obtaining data on the deep interior of the Moon. This work suggests that, if the free vibrations are excited, an ultra-long period seismometer or tidal gravimeter designed to record periods up to 16 min. should provide more detailed information.

Acknowledgments for IBM 7090 computing time are due to the National Aeronautics and Space Administration, Theoretical Division, and International Business Machines Corporation, Watson Scientific Computing Laboratory at Columbia University.

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PHYSICS

Cæsium-137 Fall-out over Norway since September 1956

In an earlier communication[1], we reported that concentrations of radioactivity in the lower stratosphere over south-east Norway had apparently increased for a month or two after high-yield test explosions, and then decreased with an effective half-life of about one month, corresponding to a residence half-life of about three months. Concentrations of radioactivity in the lower troposphere appeared to have increased for about six months, before decreasing at about the same rate as in the lower stratosphere. We suggested that deposition of fall-out materials from many explosions may have been fairly continuous, lasting months instead of years, and that during periods when explosions had

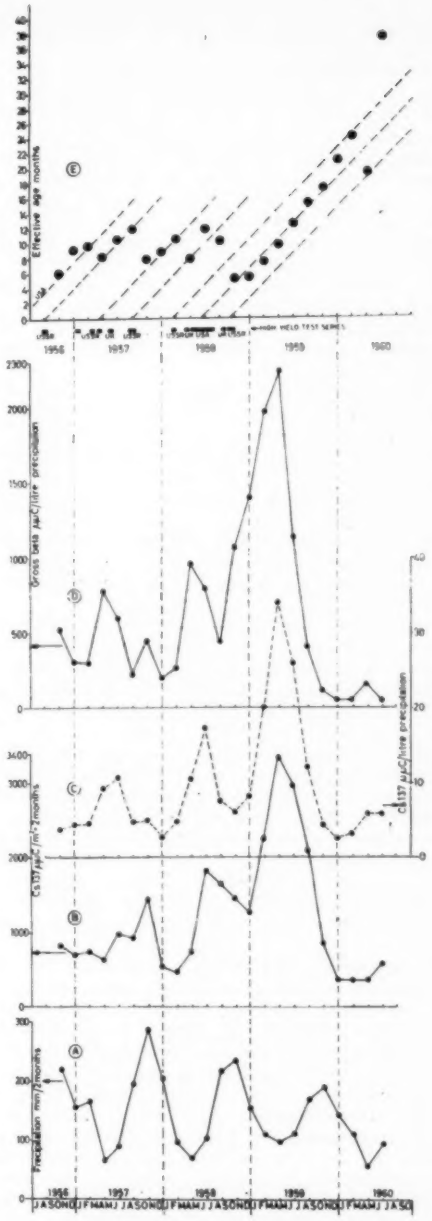
Effective age months
1956
1957
1958
1959
1960
Gross beta μμC/litre precipitation
Cs137 μμC/litre precipitation
Cs137 μμC/m²/2months
Precipitation mm/2months
J A S O N D J F M A M J J A S O N D J F M A M J J A S O N D J F M A M J J A S O N D J F M A M J J A S

Fig. 1. Fall-out over Norway since September 1956. Curve *A*, precipitation, mm./2 months; curve *B*, cæsium-137/m.² 2 months; curve *C*, cæsium-137/l. precipitation, averaged over periods of 2 months; curve *D*, gross β/l. precipitation, averaged over periods of 2 months; curve *E*, effective age of fall-out materials, months. The points are plotted at the end of the 2-month periods. Each point represents an average value of measurements from ten stations

taken place rather frequently, there may have been considerable mixing of fall-out materials from different explosions.

These conclusions were based mainly upon measurements of gross β-activities. We have also investigated the problem by measuring concentrations of cæsium-137 in the same precipitation samples, using a γ-spectrograph. The results are shown in Fig. 1 as mean values for each period of two months between September 1956 and June 1960. Curve *A* shows the mean values of precipitation; curve *B* the mean values of cæsium-137 activity deposited per m.²; curve *C* the mean values of cæsium-137 activity per litre of precipitation; curve *D* the mean values of gross β-activity per litre of precipitation.

Curves *C* and *D* are fairly alike with the main differences appearing shortly after large injections of fall-out materials.

Generally speaking, deposition of cæsium-137 activity over Norway appears to have followed the same sort of pattern as the overall deposition of β-emitting isotopes. The rather rapid decrease of deposition during the last eight months or so of 1959, following a pattern visible on previous occasions for shorter periods, has not continued into 1960. However, it is questionable whether the change is of great significance in the fall-out of materials injected into the lower stratosphere during the latter part of 1958.

From information communicated by Dr. E. A. Martell, materials from high-yield, high-altitude injections during 1958 may not have reached the lower stratosphere in significant concentrations before about the beginning of 1960. Another factor which must be borne in mind in considering the 1960 values shown in curves *C* and *D* is that although the values are shown as activities per litre of precipitation, deposition without precipitation is also included. In relatively dry periods the activity of surface air tends to increase, with a resulting increase in the rate of dry deposition and in activity per litre of precipitation. Certain variations in activity per litre of precipitation (curve *C*) may therefore be partly due to the seasonal variations in the amount of precipitation (curve *A*).

By comparing cæsium-137 activities with gross β-activities, it is possible to obtain some idea of the effective ages of deposited materials. The accuracy is limited because of the difficulties of calibrating β-counting equipment when measuring bomb debris of different ages and origins, and also because of uncertainties about the yields of cæsium-137 relative to other radionuclides, and uncertainties about possible fractionation effects. Nevertheless, as shown in curve *E*, there is reasonably good evidence from cæsium-137/gross β-activity ratios for the general conclusion that fall-out materials from many high-yield test series have reached the lower troposphere within a few months and been deposited rather rapidly.

The preponderance of materials from particular insertions during different periods is well illustrated. From about the end of 1959, however, there appears to have been considerable mixing.

In conclusion we would like to suggest again that a large part of the fall-out from many test explosions has been rather continuous over months instead of years.

There has been no outstanding seasonal variation in the rate of fall-out which cannot be related to the 'seasonal variation' in test explosions and seasonal variation in the amount of precipitation.

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¹ *Nature*, **185**, 805 (1960).

New Delayed-Neutron Precursors

With the exception of a few cases (lithium-9, nitrogen-17 and thallium-210) all delayed neutron precursors so far identified[1] have been found among nuclides with two or more neutrons in excess of the major closed shells (50 and 82). The theory of delayed neutron emission probabilities given by Pappas[2] and the subsequent general systematics of delayed neutron precursors developed by Pappas and Rudstam[3] predict, however, that delayed neutron emission should be much more common among neutron-rich nuclides than had been anticipated earlier. Delayed neutron precursors are now expected to appear from the lightest to the heaviest nuclides on the neutron-rich side of the β-stability line. The distance from it in *Z* should be from about 3 to about 7 charge units, depending on the neutron number[3]. A search was therefore made experimentally for some of these precursors.

Because of the complex mixture of delayed neutron activities already found in fission[1], an approach via fission would not be easy. Chemical separations should also be avoided because of the short half-lives expected. A feasible way of studying the prospective delayed neutron precursors would, however, be by the way of spallation reactions. These give products in highest yields near the target nuclide, whereas yields of distant products depend mainly on the irradiation energy. Therefore, by choosing suitable target elements and bombarding energies, it should be possible to detect and probably also to localize eventual delayed neutron precursors.

The short half-lives and the low neutron yield (the latter caused by the rather small branching ratio to the neutron-emitting state of the delayed neutron emitter) necessitate that the measurements start immediately after irradiation. This requirement was fulfilled by placing the neutron detector at a small radial distance from the irradiated target in the vacuum chamber of the synchro-cyclotron.

The neutrons emitted were thermalized by a layer of paraffin between the target and the neutron-sensitive scintillator properly shielded from stray radiation. The light pulses were fed through a light-guide to a photo-multiplier outside the fringing field of the magnet. The pulses were amplified and fed to a fast scaler provided with a printer. The counting started immediately after the end of irradiation.

A possible source of error in such measurements could be introduced by counting γ-rays from spallation products. The sensitivity of the detector for γ-rays is many orders of magnitude lower than for neutrons, but the intensity of the γ-rays involved is much larger than the intensity due to the neutrons.

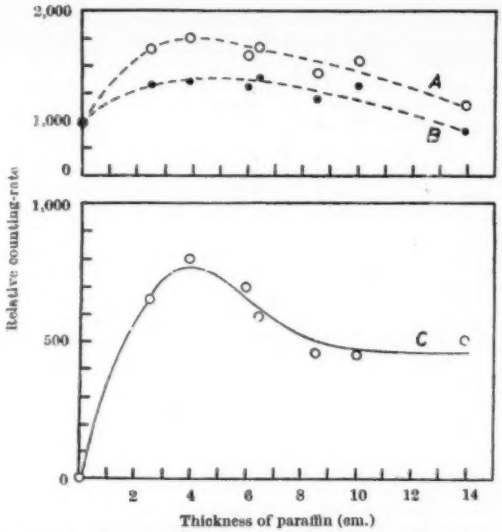

Fig. 1. Influence of paraffin and cadmium shielding on the detecting efficiency of the neutron scintillator. Curves *A* and *B* were taken without, and with, cadmium, respectively. Curve *C* gives the difference between *A* and *B*

Therefore, to make certain that only neutrons were counted, experiments were carried out using various amounts of paraffin under otherwise identical irradiation conditions. A typical curve obtained for a copper target (15 mm. 'radial thickness') is shown in Fig. 1 (curve *A*). In this figure the corresponding curve obtained with 2 mm. of cadmium between the paraffin and the scintillator (curve *B*) is also shown.

Even if Fig. 1 shows that the majority of the counts obtained with cadmium shielding are due to neutrons (the detector is also sensitive to fast neutrons, although the sensitivity for thermal neutrons is about 20 times larger), it was found appropriate to take decay curves with and without cadmium under otherwise identical experimental conditions, and then analyse the difference curve. Thus any possible contribution from γ-rays is completely eliminated. The optimum condition regarding the amount of paraffin was found by taking differences between curves *A* and *B* in Fig. 1. These are given in curve *C* as a function of the thickness of paraffin.

In Fig. 2 analyses of decay curves obtained from irradiations of radially thick copper and neodymium targets are given. Apparently the copper difference curve 3 can be resolved into several components. A peeling-off analysis discloses two components with half-lives about 18 sec. and 8 sec., and in addition a third short-lived one.

The curve obtained by irradiation of uranium was too complex to be analysed, but the total neutron activity was about five times larger than for copper under the conditions used.

Preliminary runs with neodymium (Fig. 2) disclosed activities with half-lives of about 20 sec. and 6 sec. These should presumably be due to the known[1] delayed-neutron precursors, 24·3-sec. iodine-137 and 6·3-sec. iodine-138.

It might be argued that the neutrons observed in these experiments are due to (γ,n) reactions induced in the targets or surrounding material by γ-rays from spallation products. The thresholds, however, for possible photo reactions are greater than 10 MeV.[1]. Such energies are unlikely to be present in radioactive decay of spallation products, except for those having extremely low yield (far from stability). Moreover, the (γ,n) cross-sections involved are very small at these energies. Using very conservative estimates, about 1 of every 10^4 neutrons observed might be due to a photo neutron. Thus the activities found in copper are due to hitherto unknown delayed-neutron precursors.

Since the target itself moderates and to some extent absorbs the emitted neutrons, depending on its thickness in the radial direction toward the detector, it was found that the 18-sec. activity was suppressed, and only the 8-sec. activity appeared, when using a thin copper target. The opposite occurred when placing a cadmium sheet between a thick copper target and the paraffin. This filtering is caused by difference in kinetic energy of the delayed neutrons involved.

The new delayed-neutron precursors have not yet been identified by mass and charge, and the analysis of the decay curves might be more complex than assumed. Nevertheless, these findings give strong support to the validity of the delayed-neutron precursor systematics in the earlier papers[2,3] and

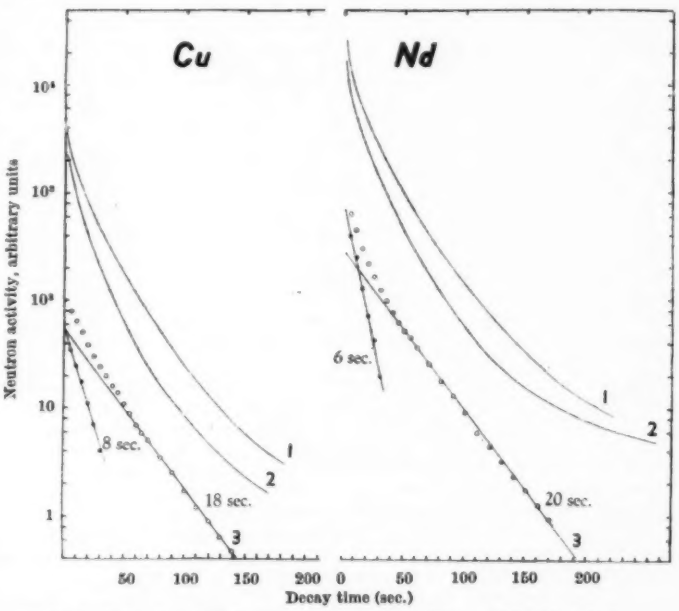

Fig. 2. Delayed-neutron decay curves. Curves 1 and 2 were obtained without, and with, cadmium, respectively. Curve 3 is the difference curve. Curves are shown for a thick copper target + 3·9 cm. paraffin and for a thick neodymium target + 3·9 cm. paraffin. (The difference curves are lower by a factor of 10 on the scale of the graph)

to the hypothesis of the existence of many delayed-neutron precursors[2]. These experiments are therefore being extended to other elements of the Periodic System.

A detailed description of the apparatus used will be published elsewhere, as well as additional results, including attempts to identify the delayed-neutron precursors by different approaches.

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Natural Occurrence of Samarium-146

Samarium-146 was discovered in 1953 by Seaborg and Dunlavey, who produced the nuclide by an intense bombardment of neodymium with α-particles[1]. They reported that it emitted α-particles of 2·55-MeV. energy and decayed with an α-half-life of approximately 5×10^7 years.

Macfarlane and Kohman made a search for samarium-146 α-activity in natural samarium employing a large internal-sample cylindrical ionization chamber[2,3]. They obtained a negative result and set an upper limit of 0·01 disintegration/gm./sec. for the specific activity in natural samarium.

A report has recently been made by Vorob'ev, Komar, Korolev and Solgakin of an indication for the existence of samarium-146 α-particles in the natural samarium α-spectrum although the level observed was not considered by them to be statistically significant[4]. They established an upper limit of 0·03 disintegration/gm./sec. for the specific activity.

The purpose of this work was to increase the sensitivity over previous measurements for the detection of a natural samarium-146 α-activity by counting a sample 'enriched in samarium-146'. This sample was obtained from the mass 146 position of a calutron collection plate which had been used in the separation of macro amounts of samarium isotopes from natural samarium.

The samarium oxide was deposited on a stainless-steel sheet and placed in an internal-sample cylindrical ionization chamber similar in design to that used previously by me[2]. It measured 50 cm. in length and 14 cm. in inside diameter, providing an active sample area of 2,000 cm.². The collecting electrode was a 0·13-mm. stainless steel wire strung axially along the full length of the counter and operated near ground potential. A negative potential of 1,400 V. was applied to the sample backing. The counter was operated as a flow-counter at 1 atm. pressure with a gas mixture of 93 per cent argon and 7 per cent methane.

Electronic amplification was provided by a 'ball and chain' preamplifier and a 'Miemar' main amplifier

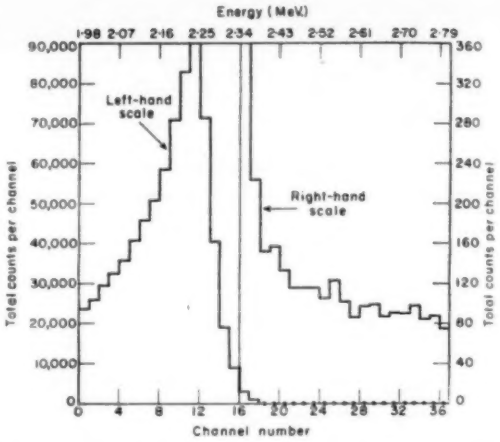

Fig. 1. α-Particle spectrum of 'enriched samarium-146' sample

(designed and fabricated at the Lawrence Radiation Laboratory, Berkeley, California). The spectrum was recorded on a 100-channel pulse-height analyser (manufactured by Pacific Electro-Nuclear Corporation, Culver City, California).

An 89-mgm. sample (approximately 45 μgm./cm.²) of the samarium oxide 'enriched in samarium-146' was counted for a period of 64 hr. The α-particle spectrum obtained is shown in Fig. 1. Only the samarium-147 peak was prominent; there was no significant indication of the presence of second peak in the vicinity of 2·5 MeV.

It was estimated that a peak in the region between 2·4 and 2·7 MeV. would have been recognized had one channel been 20 counts higher than the neighbouring channels for the 64-hr. period. From the spectra of natural samarium samples of similar thickness, it was found that the channel in which the main portion of the α-peak was contained accounted for 3 per cent of the total disintegration rate. From these data, a limit of 0·03 disintegration/gm./sec. was established for the specific activity of samarium-146 in this sample.

On comparison of the samarium-147 specific activity in the 'enriched samarium-146' sample with that for naturally occurring samarium, it appeared that an enrichment of 1·7 was realized for samarium-147. Based on the mass analyses of other samples which had been mass-separated under the same conditions, an enrichment of 1·7 in an adjacent mass (samarium-147) would correspond to at least a factor-of-10 enrichment in the isotope of interest (samarium-146), provided it had been present initially in low isotopic abundance.

In terms of the specific activity for an equivalent natural samarium sample, then, the limit would be smaller by a factor of 10 than for the 'enriched samarium-146' sample, or 0·003 disintegration/gm./sec.

Using the value of the half-life of 5×10^7 years obtained by Seaborg and Dunlavey[1], an upper limit

Table 1. Summary of Results on the Specific Activity of Samarium-146 in Nature

Investigator	Specific activity (disintegration/gm./sec.)
Vorob'ev *et al.* (ref. 4)	< 0·03
Macfarlane and Kohman (refs. 2 and 3)	< 0·01
This work	< 0·003

for the isotopic abundance of samarium-146 can be calculated from the results obtained here. An upper limit of 2×10^{-7} per cent for the isotopic abundance of samarium-146 in natural samarium was calculated. The best mass spectrometric limit, obtained by Collins, Rourke and White, is 8×10^{-5} per cent (ref. 5).

Recently, the α-particle energy of samarium-146 (produced by bombardment of neodymium with α-particles) was rechecked and found to agree exactly with the value Seaborg and Dunlavey originally reported (2·55 MeV.) (M. C. Michel and R. D. Macfarlane, Lawrence Radiation Laboratory, unpublished results). From theoretical considerations based on this energy, an α-half-life in the neighbourhood of 6×10^{7} years is favoured. If this is the correct half-life, all primordial samarium-146 should now be neodymium-142.

The co-operation of the Oak Ridge National Laboratory in specially preparing the samarium sample is gratefully acknowledged. This research was supported by the U.S. Atomic Energy Commission.

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Radiation-induced Defects in Lead Silicate Glass

IN attempts to explain the process of coloration of glass by γ-radiation, it was suggested in a previous communication[1] that electrons are trapped in defects to form colour centres. Not only do these defects already exist in glass but they can also be created by exposure to radiation. If defects could not be so created, it would be expected that the concentration of colour centres, as measured by the optical density, would increase exponentially to a saturation value at a certain dose. In practice, it is found that the optical density increases exponentially with dose up to about $10^{5 \cdot 5}$ rads, but thereafter the increase is linear. This effect is shown in Fig. 1, which is divided into two sections having different scales for dose, the first section illustrating the exponential portion and the second the linear portion of the curve.

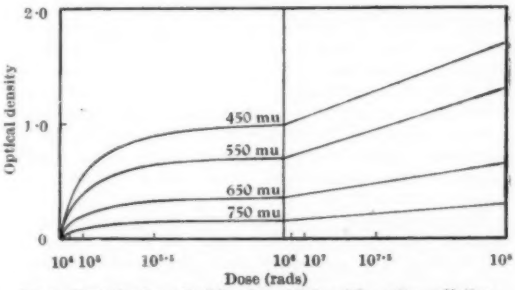

Fig. 1. Variation in optical density with dose (time after radiation, 1,000 min.)

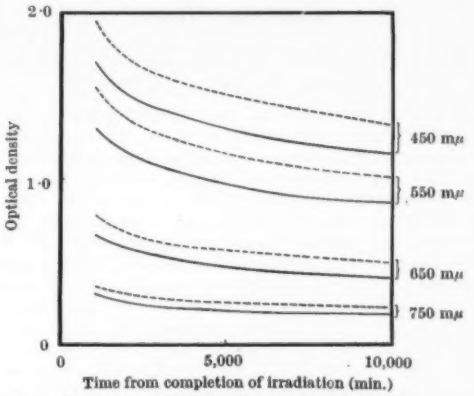

Fig. 2. Increase in optical density on second irradiation. ——, first irradiation; – – –, second irradiation after bleaching

The linear portion is attributed to the creation of defects by the radiation. Thus the extrapolation of the straight line to zero dose gives an indication of the defects already present in the glass. The difference between the extrapolated value at zero dose and the measured optical density is proportional to the concentration of defects produced by the radiation.

Exposure of irradiated glass to light at room temperature produces bleaching of the colour centres by excitation of the trapped electrons to such energy-levels as to enable them to escape from the defects and recombine with the positive holes. It might be expected that such treatment should release the trapped electrons but have little effect on the total number of defects. Hence such a bleached sample of glass should contain a greater concentration of defects or trapping centres than an unirradiated sample and, on further irradiation, should contain more colour centres than an originally unirradiated sample which is subjected to a first irradiation.

To test this theory a sample of the lead silicate glass used in the experiments described previously was given a dose of 10^{8} rads of γ-radiation and its optical density was measured over the wave-length range 360–1,000 mμ at intervals of time during the week following the irradiation. The induced coloration was then bleached by exposure to light from a mercury lamp until the optical density of the glass was not significantly different from that measured before the irradiation. The sample was then given a further dose of 10^{8} rads, and the optical absorption was again measured.

Fig. 2 shows the variation in optical density with time for four wave-lengths after the first and second irradiations. It can be seen that the optical density of the sample after bleaching and re-irradiation is at all times greater than that obtained after the first irradiation. This result is in accord with the proposed theory.

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ENGINEERING

Beneficial Effects of Reagents in Solution on Wet Crushing of Rock

Earlier workers in this Department[1,2] have reported that substantial increases in the crushing efficiency of a drop-weight mill (stamp mill) may be achieved by pretreatment of the ore in aqueous solutions of certain reagents.

This work appears to have aroused interest, but, in the cases known to us, workers elsewhere have not been able to obtain significantly beneficial results[3,4]. In these latter investigations a ball mill was used, and it is of interest that the first exploratory work at Leeds was carried out with a ball mill; the results were not encouraging[3]. Possible reasons for this, and for the final choice of a drop-weight mill, have been discussed by Frangiskos[2]; arguments are based on the work of Rehbinder[5].

In view of these later findings it has been considered necessary to repeat some of the experiments reported by Frangiskos and Smith[1] with the drop-weight mill. The results are given in Table 1. Except for the actual rock samples (limestone) used, the experimental conditions are the same as those employed by Frangiskos and Smith. The amount of crushing achieved is measured in terms of the surface area of the product, determined by a gas-permeability method. The percentage increase shown in Table 1 is the extra amount of crushing achieved when the ore is pretreated in a solution instead of in distilled water. The beneficial effect with the particular samples of limestone used is even more pronounced than that observed by Frangiskos and Smith; their best results represented approximately a 50 per cent increase.

Table 1. Effect of Reagents in Solution on the Surface Area of Crushed Ore

Reagent (mgm./l.)	Sodium hydroxide		Sodium carbonate	
	Surface area of product (cm.²/gm.)	Percentage increase (pure water basis)	Surface area of product (cm.²/gm.)	Percentage increase (pure water basis)
0	247*	—	156	—
100	305	23·5	185	18·6
150	367	48·6	—	—
200	346	40·1	273	75·0
300	278	12·5	296	89·8
400	261	5·7	320	105·0
500	270	13·0	293	89·0
600	301	21·9	244	56·4
700	354	43·3	217	38·2
800	282	14·2	187	19·9

Charge to mill: 15 gm. limestone (−2·05 mm. + 1·40 mm.). Conditioning time in solution, 10 min. Weight of crushing head, 2,026·4 gm. Height of fall, 13·6 mm. Blows per min., 120. Total number of blows, 600.

* Initial surface area approximately 1·5 cm.²/gm.

It is suggested that the reason for the success of the drop-weight method of crushing in contrast to the ball mill is basically as proposed by Frangiskos and Rehbinder. The essential feature of the hypothesis is that embryo microcracks produced by stress on the solid are made permanent points of weakness by adsorption in them of ions or molecules from solution; it is assumed that the relative adsorption of water molecules is so small as to permit resealing of the microcracks. A drop-weight mill is likely to be more effective than a ball mill in producing microcracks.

Furthermore, the choice of method for assessing the amount of crushing (fineness of product) is important. Most of the new surface area produced is contained in the extremely fine sizes[6], and the conventional sieve analysis gives no measure of this. Results which have been assessed in terms of a sieve analysis might therefore indicate no beneficial effect of reagents in solution. The work in this Department was terminated in 1956 after three years work due to lack of financial support. Finance has now been made available for the project for one more year, but it seems to us a pity that further development work on the process may have to be carried out elsewhere.

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Hydrogen Diffusion in Water-accelerated Rolling Surface Fatigue

The pitting failure of rolling bearings is now generally regarded as a surface fatigue phenomenon, and recent work[1] has shown that the presence of water in mineral oil lubricants can accelerate the failure of ball-bearings manufactured from conventional *E.N.*31 steel. A possible mechanism for this phenomenon has been suggested by Grunberg and Scott[2]. The hypothesis is that vacancy-induced diffusion of hydrogen into the highly stressed surface material produces 'hydrogen embrittlement'. To test this theory, four-ball rolling tests[1,2] were carried out with a lubricating oil containing 6 per cent tritiated water of high activity (5 c./ml.). The rolling four-ball test produces a track on the driving ball in which pits are normally formed. The three driven balls are stressed to a lesser extent and usually do not show evidence of surface fatigue. Balls from tests using tritiated water were washed with acetone and partially immersed in a liquid scintillator (0·3 per cent 2,5-diphenyloxazole + 0·01 per cent 1,4[2-(5-phenyloxazolyl)]-benzene in toluene) and the activity counted using a (refrigerated) ECKO *N*612 unit. When the part of driving balls carrying the fatigued track was immersed in the liquid scintillator, counting-rates of between 1,000 and 2,000 counts/sec. were recorded. The unfatigued part of driving balls gave only counts of about 40 counts/sec. against the background count of 30 counts/sec. The activity in the fatigued tracks decreased with time and tended towards the background-level after about five days.

The preliminary experiments described provide support for the hypothesis that, in the presence of water, hydrogen penetrates into the material subject to surface fatigue. The decrease in activity with time can be ascribed to escape of tritium from the material.

The phenomenon is now being investigated in greater detail.

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METALLURGY

Fatty Acids and Adhesion of Electrodeposited Metals

The deleterious effect of grease on the substrate on the adhesion of electrodeposited metals is well known, but quantitative data are sparse. A systematic study of the influence of monolayers of adsorbed surface-active agents on the adhesion of electrodeposits is in progress in this laboratory, and this communication reports the initial results obtained from the fatty acid series.

The adhesion was measured in the manner described by Williams and Hammond[1], with the test-pieces made of copper. The ends of the specimens to be plated were polished to 1μ diamond powder, electropolished in 50 per cent phosphoric acid and treated to have a monolayer of the fatty acid under test[2]. The acids used were the even-numbered acids of the series $CH_3(CH_2)_nCOOH$, where $n = 4$–16. The specimens with the monolayer were plated with nickel from a conventional Watts type solution at a *p*H of 4, temperature of 40° C., and a current density of 20 amp./sq. ft. until a deposit of at least 0·1 in. had been produced. After plating, the specimens were tested in a Denison tensile testing machine. To provide a reference point, a series of specimens was tested using the exact procedure outlined except that no fatty acid was used.

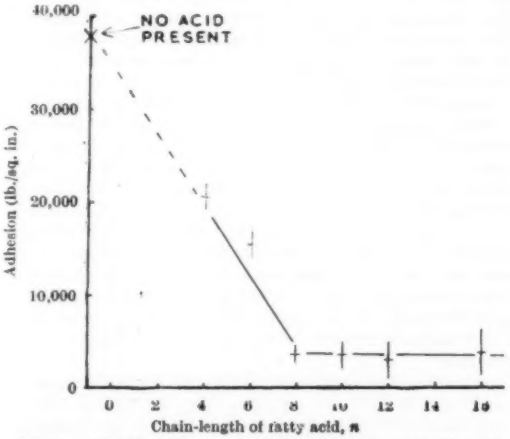

Fig. 1. Adhesion of electrodeposited nickel as a function of fatty acid chain-length

The results are shown in Fig. 1. They were reproducible for each acid, and when the experiment was repeated with caprylic acid ($n = 6$) the adhesion values were within a few hundred pounds per sq. in. of each other. The adhesion appears to be independent of the chain-length of the fatty acid when n is greater than 8 or 10; as n decreases below this value the adhesion progressively increases. This increase can be explained either by the increased desorption of the shorter-chain acids resulting in the nickel being discharged directly on the copper lattice, or by a decrease in the long-range forces between copper and nickel as the distance increases. It is impossible to distinguish between these alternatives at this stage, but it may be pertinent that there is no evidence of epitaxis.

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Fatigue Straining of Copper Whisker

Brenner[1] has shown that copper whiskers can be strained elastically to as much as 2·8 per cent in tension. I have found that similar whiskers remain elastic in cantilever bending at static strains up to 4 per cent corresponding to a stress of 470 kgm./mm.$^{-2}$ in a whisker of $< 110 >$ orientation.

When plastic deformation occurs it is by 'kinking', as has been described by previous workers[2] with other materials. The 'kink' formed is a bend of radius of curvature of the order of the thickness of the whisker; it is produced suddenly, with release of the elastic strain in the rest of the whisker. It can be shown that this behaviour is to be expected from a cantilever bent by a 'hard' device, if it is of a material that has clearly defined upper and lower yield points and yield extension. These phenomena are very pronounced in copper whiskers[3].

I have performed experiments to see whether the behaviour of copper whiskers under repeated straining differs from that under static conditions. The experiments were performed in cantilever bending with the whisker encased in diphenyl carbazide glass[4] and deflected by a 'hard' probe. The whiskers were grown by hydrogen reduction of cuprous iodide[5].

Since the static strain at which kinking occurs varies very much from one whisker to the next, it was not possible to test whiskers in cyclic loading at a known fraction of the static kinking strain. Instead, whiskers that were less than about 3μ thick, straight and optically smooth, were selected and were then tested as follows.

A whisker was bent to a strain, S ($\simeq$ 1 per cent), which it was thought very likely to be able to sustain elastically. The bending-probe was then removed, and the whisker examined for permanent bending. If there were none, the probe was then made to vibrate so that the maximum strain in the whisker fluctuated between 0 and S at 900 c./s., the test being observed stroboscopically. After 10^6 cycles of vibration the probe was stopped and removed. If no permanent deformation had taken place, a static strain of $(S + \Delta S)$ ($\Delta S \simeq 0{\cdot}3$ per cent) was applied, and the whole procedure repeated, the vibrations being this

time with maximum strain fluctuating between 0 and $(S + \Delta S)$. This procedure was repeated with successive increments of strain. Thus the whisker was subjected, at each value of strain amplitude, to one slow (quasistatic) cycle of strain, followed, if nothing had happened, by 10^6 fast cycles.

I found that whiskers were generally capable of surviving such tests apparently unaffected to strains of the order of 3 per cent, with 10^6 strain cycles at each value of strain amplitude. Then, during the next period of oscillatory straining, at a slightly higher strain, the whisker would suddenly kink, after it had already been subjected to a large number of cycles at this high strain. The kinking would be instantaneous, so far as could be judged by eye; that is, it must have happened in less than 100 cycles. The vibrator would reach maximum amplitude in at most a few hundred cycles, and kinking was observed less than a few thousand cycles after the increment of strain was made. Several times the kinking occurred after more than 10^5 cycles.

The strains at which whiskers kinked dynamically were not very reproducible, just as the static kinking strains show a large scatter[1]. It sometimes proved possible to re-embed a whisker that had kinked dynamically in such a way that the undeformed part of the whisker could be re-tested. The strain at which dynamic kinking occurred was then of the same order as in the first experiment. For example, a whisker that had kinked after being strained from 0 to 1·9 per cent for 10^5 cycles was re-embedded; it afterwards withstood 10^5 cycles at 0–1·1 per cent and 10^5 cycles at 0–1·7 per cent strain and it kinked after 10^6 cycles at 0–2·3 per cent strain.

If the vibrator was stopped immediately after the whisker kinked, then the whisker looked just as if it had kinked statically. The kink was a plastic hinge, but its appreciable strength often made it possible to continue elastic vibrations with much-reduced amplitude (perhaps 0·3 per cent elastic strain). When this was done, the whisker usually fractured in the kink after a few thousand cycles. As a corollary, if the vibrator were not stopped when the whisker kinked, fracture occurred a few thousand cycles after kinking. Fracture was never observed in oscillatory loading of this type unless kinking had occurred first.

If the whisker was fixed both at the root and to the vibrator (instead of merely being pushed by the latter) and vibrated so that the strain oscillated symmetrically about zero, then fracture did occur. But in this case kinking was not easily detectable, and, if it had taken place, the plastic kink would have been subjected to large cyclic strains, so that it would have failed very quickly, perhaps too quickly to see the kinking. Therefore this test does not provide adequate evidence of the existence of fatigue fracture in elastic whiskers.

Whiskers were also statically strained for periods long compared with the times of the oscillatory experiments. Kinking was never observed unless it happened immediately the strain was applied. This shows that the delayed kinking is due to the oscillatory loading and not to the passage of time.

Both statically and dynamically kinking occurs near the root of the whisker, where it is embedded in diphenyl carbazide glass and where the bending moment, and hence the elastic strain before kinking, are greatest. But the kink very often starts, not at the point of entry of the whisker into the glass, but at distances up to ten times the thickness of the whisker away from that point, towards the free end. Therefore, the failure of the whisker is not determined by stress concentration or corrosion at the point of entry into the glass: the concentration is probably small, owing to the low elastic modulus of the glass compared with that of the copper, and to the wetting-fillet, the radius of which is of order ¼–½ the whisker thickness. The failure is more probably precipitated by stress concentration at defects in the whisker[6].

There is much evidence[6] that in copper whiskers subject to large, static, elastic strain, no slip occurs below the yield point. We have shown that if they are subjected to large, repeated, elastic strain, fatigue fracture is not observed, even at strains of the order of 3 per cent. The sudden onset of plastic flow does occur; the reason for this is not understood, and is being investigated. As soon as the whisker has flowed plastically, it can fracture in fatigue, in the yielded region, at quite small, mainly elastic strains. This serves to confirm the belief, hitherto assumed without direct evidence, that slip is an essential process in fatigue failure.

I wish to thank Mr. H. B. Holland, who has carried out these very delicate experiments. This communication is published by permission of the Ministry of Power.

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RADIOCHEMISTRY

Effect of γ-Radiation on Nisin

The antibiotic nisin is known to reduce the heat-resistance of spore-forming organisms, and consideration has been given to its use as an additive to lower the heat-process requirements of canned foods[1]. The radiation resistance of spore-formers might also be influenced by nisin, and the dose required for the sterilization of food lowered to a level where radiation-induced flavour and odour changes are not detectable.

Experiments were first conducted to determine the effect of radiation on the antibiotic activity of nisin when it was suspended in a variety of media and at different *p*H values. Samples were made up in McCartney bottles in triplicate and irradiated with γ-rays at a dose-rate of 1×10^5 rads/hr. using a 10,000 c. cobalt-60 source. The temperature of the samples was maintained at 4° C. during irradiation and until assay, 30 hr. after their preparation. Nisin was assayed using both the resazurin[2] and plate methods[3], the latter being modified to handle liquid samples.

The result of irradiation with a dose of 2·7 megarads is shown in Table 1. At this dose-level nisin activity is completely destroyed irrespective of the nature of the suspending medium or of the *p*H in the short

Table 1

Media	Nisin concentration (U./ml.) mean of triplicates			
	Theoretical	Before irradiation	After 30 hr. storage (controls)	Irradiated 2·7 megarads
Sterile skim milk	200	220	190	< 4
Sterile skim milk	2,000	1,000	1,750	< 4
Unprocessed soup *p*H 6·08	200	—	238	< 4
Processed soup *p*H 5·85	200	—	180	< 4
*p*H 3·5 phthalate buffer	200	175	170	< 4
*p*H 5·0 phthalate buffer	200	235	200	< 4
*p*H 6·0 phthalate buffer	200	200	205	< 4
Pure nisin in sterile distilled water	2,000	2,250	2,250	< 4

range studied. No activity could be detected even when the initial concentration of nisin was increased from 200 to 2,000 U./ml. as shown in skim milk and distilled water. No significant loss of unirradiated nisin occurred during the 30-hr. period of the experiments. The soup medium used was a meat and vegetable broth chosen to demonstrate any possible protective effect which might be exerted by food constituents.

Sterile skim milk with an initial concentration of 200 U./ml[1]. of nisin was chosen as suspending medium to study a range of radiation dose-levels. The experiment was run in triplicate and results are shown in Table 2. At the lower levels about 17 per cent of activity is lost, there is a marked drop at 0·5 megarad when 80 per cent is lost and a complete loss occurs at 1·5 megarads and above.

Table 2

Dose (megarad)	Nisin concentration (U./ml.) mean of triplicates	
	Resazurin method	Plate method
Nil (control)	183	182
0·13	153	152
0·25	150	153
0·5	37	24
0·75	< 20	22
1·0	< 60	14
1·5	< 60	< 5
2·0	< 60	< 5
2·5	< 20	< 5

The evidence presented indicates that nisin added to foodstuffs prior to radiation processing will be rendered ineffective against bacterial spores which survive the radiation treatment, especially in the sterilization dose-range of 1–3 megarads. There is a possibility, however, that nisin might be protected from radiation damage to some extent in the presence of spores, particularly if it acts by absorption on the spore coat.

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CHEMISTRY

Electron Paramagnetic Resonance Studies of Stable Free Radicals in Lignins and Humic Acids

Electron paramagnetic resonance studies[1] of wood ; lignins ; tannins, and humic acids, such as are found in soils and coals, show an electron paramagnetic resonance line of 6 ± 2 gauss width at $g = 2{\cdot}003 \pm 0{\cdot}002$, indicating the presence of a free radical. I find that this free radical is stable over a period of years in air and is not present in protolignin in plant materials nor is it necessarily the result of simple ageing or rotting of wood. However, it is formed when protolignin in plants is polymerized into lignin by either acid or fungal attack. I also find that the 6 ± 2 gauss electron paramagnetic resonance line is restricted to the fraction of humic material which is extractable with aqueous bases.

Freudenberg[2] indicates that lignin polymerization results from dehydrogenation or oxidative removal of a phenolic hydrogen on coniferyl or sinapinic alcohol or by hydrolysis of a phenolic glycoside linkage. According to Freudenberg the polymerization is a free-radical process involving a semi-quinone radical intermediate. My electron paramagnetic resonance results indicate that free radicals are indeed present and that some free radicals persist in the lignin polymer where they are protected from their macro-environment. They survive within the lignin micelles through the process of coal formation and persist apparently for geological time ($\sim 10^8$ years).

Air-dried fresh wood samples (fine shavings) of oak, pine, eucalyptus, redwood, and spruce showed a very low free radical concentration of $< 10^{14}$ spins per gram (s./gm.). The carbon disulphide-soluble, absolute ethanol-soluble, and dioxane-soluble Soxhlet fractions (extracted in this order) and the corresponding residual wood fractions showed no electron paramagnetic resonance ($< 10^{14}$ s./gm.). Dioxane (catalysed by hydrochloric acid) lignin extracts showed strong electron paramagnetic resonance $\sim 10^{18}$ s./gm. at $g = 2{\cdot}00$ even though the lignin precursors in the wood did not. A similar high free-radical content was observed with a 'Soxhlet' water extraction that turned acid by formation of organic acids from the wood. Electron paramagnetic resonance was not evident in the unfractionated cellulose and pentosan fractions of wood. Freshly cut redwood ranging from sapwood to rotted heartwood (40 years old) showed no difference in a very weak electron paramagnetic resonance signal. Scots pine buried for 600 years in damp soil, and spruce buried for more than 450 years[3] likewise showed no, or barely detectable, electron paramagnetic resonance. These same woods showed depletion of cellulose and pentosans, but no change of lignin concentration. On the other hand, fresh pine needles about to drop from a tree but already attacked by fungi, and plant debris which had collected in soil as peat showed strong electron paramagnetic resonance with both a line of 8 gauss and a broad line of 1,000–1,200 gauss both centred on $g = 2{\cdot}00$ (Fig. 1*a*). The material showing the sharper electron paramagnetic resonance line is extractable with an aqueous base (Fig. 1*b*) and behaves as the polyelectrolyte humic acid while the broad line (Fig. 1*c*) characterizes the non-extractable residue. Tannin extract (quebracho) showed the same 6-gauss

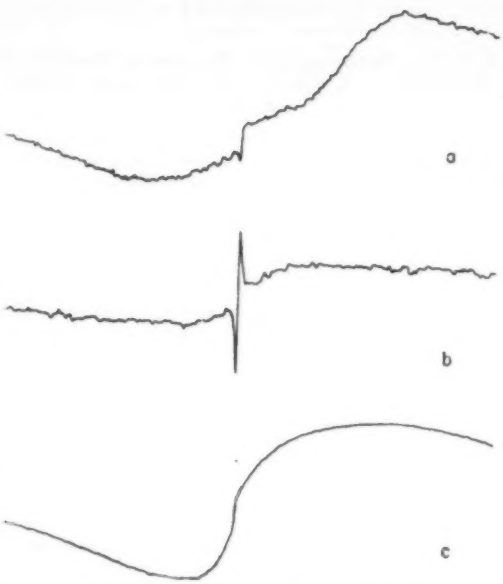

Fig. 1. Electron paramagnetic resonance of free radicals in post-Pleistocene peat from Point Barrow, Alaska (grass–sedge–moss flora). *a*, Fresh peat; *b*, *N* sodium hydroxide extract, precipitated with hydrochloric acid, washed and air dried; *c*, peat residue after extraction

electron paramagnetic resonance line shown by humic acids.

These results suggest that lignin does not normally occur in living plants. Rather it is the polymerization product of those compounds capable of forming or reacting with semiquinone free-radicals formed during dehydrogenative or hydrolytic degradation of plant tissue. These polymers with their trapped free radicals in turn survive the geochemical processes of humification and coalification without loss of their free radicals. This suggests that post-depositional chemical modifications do not destroy the aromatic semiquinoid properties of these compounds.

It should be remembered that not only aromatic —OH groups but also aromatic —NH_2 [4] and aromatic —SH [5] groups can be dehydrogenated to yield semiquinone-type radicals. For this reason a wide range of compounds can be expected to contribute to the formation of natural semiquinone polymers such as lignins, humic acids and tannins.

The comments and assistance of my colleagues and the research assistants at this Laboratory have been both helpful and stimulating. Especial appreciation is due to Dr. R. R. Unterberger for providing the facilities of his electron paramagnetic resonance spectrometer.

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Surface Renewal and Molecular Diffusion in Interphase Mass Transfer

In 1953 Lewis and Pratt[1] described violent spontaneous movements of the liquid–liquid interface which they observed during measurements of interfacial tension in the presence of mass transfer by the drop-weight method. These phenomena have since been investigated more fully[2–5]. It is now generally accepted that they are due to the formation of local gradients of interfacial tension caused by local variations of solute concentration at the interface as the result of mass transfer.

It can be argued that such spontaneous 'interfacial eruptions' would cause considerable portions of the interface to be replaced by fresh liquid from the interior and thus result in an increase of the rate of surface renewal. This could mean that for solutes of different interfacial properties the rates of mass transfer may be different even under conditions of equal flow parameters of the bulk phases and equal molecular diffusivities. No conclusions can thus be drawn from such experiments about the effects of molecular diffusivity on the transfer process. If, however, the transfer of two solutes takes place simultaneously, that is, under identical interfacial as well as hydrodynamic conditions, some conclusions could be arrived at. In order to test this possibility, experiments were conducted on the separate and simultaneous transfer of propionic acid and phenol between carbon tetrachloride and water, using vertical and horizontal laminar co-current liquid–liquid contactors with moving interface.

From the theory of mass transfer with surface renewal[6] it can be shown that for the transfer of a solute A between two immiscible solvents 1 and 2 the overall resistance to mass transfer, R_A, can be expressed as:

$$R_A = (1/\sqrt{D_1} + m/\sqrt{D_2})/s_A = 1/\sqrt{(d_A s_A)} \quad (1)$$

where s is the fractional rate of surface renewal; D_1, D_2 the solute diffusivities in the two phases; m the distribution coefficient; and d an effective overall diffusivity. Thus, for the separate transfer of the two solutes A and B:

$$R_A/R_B = \left(\frac{d_B s_B}{d_A s_A}\right)^{1/2} \quad (2)$$

and for simultaneous transfer, since $s_A = s_B$:

$$R_A/R_B = (d_B/d_A)^{1/2} \quad (3)$$

The results obtained for separate transfer of phenol and propionic acid at equal Reynolds numbers could not be correlated by equation (3), thus indicating that for the two cases the rates of surface renewal were different. Observations on simultaneous transfer, involving transfer of phenol in either direction but of propionic acid from carbon tetrachloride to water only, covering a thirty-fold range of R and a four-fold range of m_A/m_B, confirm that equation (3) holds within 95 per cent confidence limits of ± 5 per cent. In all the runs the existence of interfacial turbulence was confirmed by visual or schlieren observation. Thus it is highly probable that, under conditions of spontaneous interfacial turbulence, the rate of mass transfer depends to a large extent on the induced rate of interface renewal and is directly proportional to the square root of molecular diffusivity. Values

of fractional surface renewal up to 100 per sec. were found.

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Growth and Adhesion of Oxide in Furnace Deposits

During the oxidation of steel there are formed on the surface a variety of needle or plate-like growths of oxide which, for convenience, have been called 'oxicles'. The present investigation, supported by and in collaboration with the Thornton Research Centre of Shell Research, Ltd., began with the idea that these oxicles might entrap particles in burnt gases and thus act as the nucleation for the build-up of ash deposits. Accordingly a series of experiments have been undertaken in which specimens of 5 per cent chromium, 0·5 per cent molybdenum steel sheet have been oxidized in an oil-burning rig and examined by reflexion electron microscopy.

The results show, first, that whether or not oxicles are formed by pre-oxidation in air before the specimens are put into the oil-burning rig, the subsequent growth is similar. Secondly, there is a very large difference between the oxicle growth which occurs when the specimen is exposed to clean oil products and that obtained with oil containing a soluble metallic salt expected to give greater build-up of deposits. In the first case the growths are small, as shown in Fig. 1, whereas in the second case when oil containing a soluble metallic salt is used, large spiky oxicles occur of composition as yet unknown and these in turn do seem to collect very small particles of ash among their branches in accord with the original hypothesis (Fig. 2). Thus, although the oxicles trap some fine ash particles, this mechanism is not necessarily the dominant factor in the build-up of deposits.

The mode of attachment of the fluffy material at the tops of some of the oxicles suggested an electrical effect. The oil-burning apparatus was therefore modified so that the specimen holder was insulated from the rest of the rig and could be raised to ±3,000 V. with respect to it. Depending on the sign of the applied voltage, there was a marked difference in oxidation behaviour. In the tests with positive or zero potential, the scale was adherent, and the specimen developed a dull surface finish; but in the tests where the charge was negative, the scale was non-adherent and the metal surface retained a bright appearance. The pick-up of fine ash material did not seem to be appreciably affected. Fig. 3 shows the flaking away of the oxicle growth after oxidation with −3,000 V. applied to the specimen.

Work is proceeding to investigate these effects more fully.

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New Data for the Calcium Silicate, 'Phase Z'

This compound was first described by Roy[1,2], who considered it to be $9CaO.6SiO_2.H_2O$. Further work now shows that, while specimens prepared at relatively low temperatures and pressures may be hydrated, those prepared at sufficiently high temperatures and pressures are anhydrous, with the composition $Ca_3Si_2O_7$. They also establish the approximate unit cell parameters.

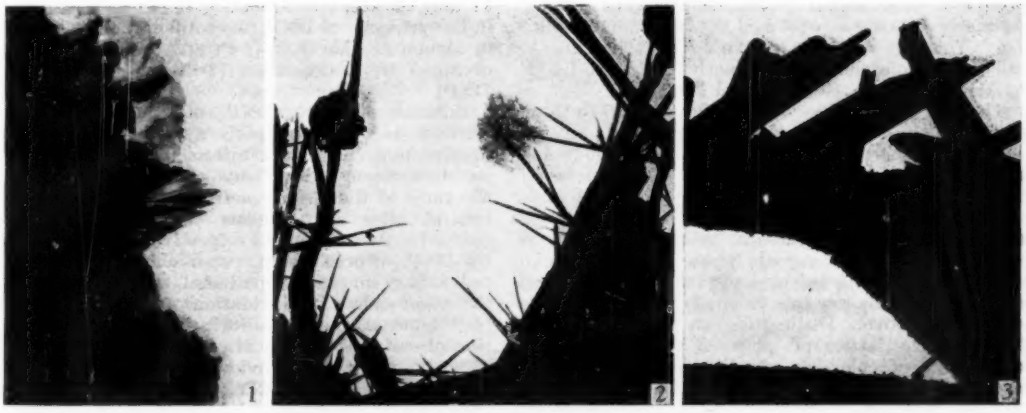

Fig. 1. Oxicle growth on 5 per cent chromium, 0·5 per cent molybdenum steel sheet after 15 min. at 600° C. in air plus 6 hr. at 600° C. in an oil-rig burning pure kerosene. (× 2,960)

Fig. 2. Oxicle growth on 5 per cent chromium, 0·5 per cent molybdenum steel sheet after 15 min. at 600° C. in air plus 6 hr. at 600° C. in an oil-rig burning kerosene containing 1 per cent of a soluble metallic salt. (× 1,480)

Fig. 3. Oxicle growth on 5 per cent chromium, 0·5 per cent molybdenum steel sheet after 30 min. at 600° C. in air followed by 6 hr. at 600° C. in an oil-rig burning kerosene containing 1 per cent of a soluble metallic salt. A potential of −3,000 V. was applied to the specimen. (× 960)

A specimen prepared hydrothermally at 715° C. and 1,100 bars showed negligible loss of weight over the range 100–1,400° C. There was no change in X-ray powder pattern up to 954° C., but by 1,090° C. transformation to rankinite had occurred. Specimens prepared under these or similar conditions are therefore anhydrous. 'Phase Z' can, however, also be obtained at temperatures at least as low as 200° C., and it seems unlikely that a product so obtained would be anhydrous. Preliminary results suggest that, as the temperature of preparation decreases, the phase becomes hydrated and that its calcium : silicon ratio rises. This can possibly be attributed to a hydrogarnet-like replacement of Si by 4H, which would cause the composition to trend towards $6CaO.3SiO_2.2H_2O$. So-called 'dicalcium silicate hydrate (C)'[2], and similar preparations, appear to be mixtures consisting principally of lime-rich 'phase Z' with smaller amounts of calcio-chondrodite ($Ca_5(SiO_4)_3(OH)_2$).

The equilibrium P—T curve for the transformation of 'phase Z' into rankinite has been reported previously[2]. Further work suggests that this curve represents a metastable equilibrium, as sufficiently long runs under conditions lying in the 'phase Z' field yield 'phase Y' ($6CaO.3SiO_2.H_2O$) and wollastonite or foshagite, with or without 'phase Z'. The latter is therefore probably always metastable, at least at the higher temperatures at which it is formed. This, together with the supposedly variable composition, may explain the difficulty of preparing large crystals : the compound disproportionates before these are able to grow. From a combination of selected area electron diffraction and X-ray powder photographs for several specimens, the unit cell was found to be orthorhombic or pseudo-orthorhombic, body-centred, with a 5·07, b 11·3, c 23 Å. approx. ; the crystals are flaky with (001) cleavage. These parameters were confirmed by X-ray study of single crystals of afwillite ($3CaO.2SiO_2.3H_2O$) which had been first heated in air at 500° C. and afterwards treated hydrothermally at 200° C. under saturated steam pressure ; these crystals contained both 'phase Z' and tobermorite, both in strongly preferred orientation. The calculated density, assuming cell contents $8(Ca_2Si_3O_7)$, is about 2·9 gm./c.c. This agrees with the value of 2·99 gm./c.c. calculated from the mean refractive index of 1·652[2] by the method of Howison and Taylor[4].

The unit cell of 'phase Z' is closely related to those of γ-Ca_2SiO_4 and calcio-chondrodite, the face with approximate parameters a 5·07, b 11·3 Å., γ 90° being common to all three compounds. We have found that 'phase Z' forms particularly readily on hydrothermal treatment of γ-Ca_2SiO_4 ; this can perhaps be attributed to a structural resemblance. It is often difficult to distinguish 'phase Z' from calcio-chondrodite by selected area electron diffraction, as in both cases the crystals normally lie on the face mentioned above. Distinction can be made by the fact that the lattice of 'phase Z' is body-centred, while that of calcio-chondrodite is primitive, and also by the differing values of c. These differences are only detectable, however, if the Laue zones corresponding to different l-indices are well resolved ; for this purpose a tilting stage technique can be used[5]. If they are not well resolved, c cannot be determined, and 'phase Z' shows an apparently primitive lattice indistinguishable from that of calcio-chondrodite.

Further work is in progress, and a fuller account will be published later.

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BIOCHEMISTRY

Different Action of 2 : 4-Dinitrophenol on the Oxidation of Exogenous and Endogenous Substrates by Baker's Yeast

It is recognized[1] that 2 : 4-dinitrophenol (DNP) stimulates the total oxidation of glucose by baker's yeast, which takes place mostly through the combined action of the Embden–Meyerhof and the citric acid cycles[2]. Nevertheless, the oxidation of pyruvate, a compulsory intermediate of glucose degradation in yeast, is strongly inhibited by DNP in the same experimental conditions that stimulate glucose oxidation[3]. In order to elucidate this apparent contradiction our previous experiments have been extended using substrates labelled with carbon-14.

Table 1 shows that with [3-^{14}C]pyruvate, DNP prevents incorporation of carbon-14 into the cells, the evolution of radioactive carbon dioxide and the oxygen uptake. On the other hand, with [6-^{14}C]-glucose (or [1-^{14}C]glucose), which yields [3-^{14}C]-pyruvate inside the cell, DNP stimulates the oxygen consumption and evolution of labelled carbon dioxide, and, accordingly, diminishes incorporation of carbon-14 into the cell constituents. It is noteworthy that in the presence of DNP, the total carbon-14 counted in carbon dioxide from [6-^{14}C]glucose is about that obtained from exogenous [3-^{14}C]pyruvate (without DNP).

Enhancement by DNP of glucose oxidation through the pentose pathway[3], which does not involve pyruvate as an intermediate, can be ruled out because in experiments carried out separately the ratio of the specific activities of carbon dioxide formed after simultaneous oxidation of glucoses marked in the C-1 and C-6 respectively is not increased by DNP. Furthermore, paper chromatograms and radioautograms of the methanol–water extracts from the yeast cells after oxidation of [3-^{14}C]pyruvate or [6-^{14}C]glucose show, qualitatively, the same distribution of carbon-14 in the citric acid-cycle and related substances. From this evidence, it may be concluded that the action of DNP on exogenous pyruvate consumption is not due to inhibition of the mechanism of pyruvate oxidation (the citric acid-cycle) but rather to diminished accessibility of pyruvate to the cell compartment where the oxidation takes place. Similar results are found with [1,4-^{14}C]succinate and [^{14}C]glutamate (Table 1) and also with [1,4-^{14}C]-fumarate.

Table 1. Effect of DNP on the Oxidation of Carbon-14-labelled Glucose, Pyruvate, Succinate and Glutamate by Baker's Yeast

Exp.	Substrate	DNP (m*M*)	Q_{O_2}	^{14}C in the yeast cells $\left(\frac{\text{counts/min.}}{\text{mgm.}}\right)$	^{14}C in $BaCO_3$ (counts/min.) ($\times 10^3$)	$\left(\frac{\text{counts/min.}}{\text{mgm.}}\right)$ ($\times 10^3$)
A	[3-^{14}C]Pyruvate, 10 m*M*	0	53·5	18,270	60·9	11·40
A	[3-^{14}C]Pyruvate, 10 m*M*	0·05	8·4	740	0·3	0·30
A	[6-^{14}C]Glucose, 5 m*M*	0	52·5	24,870	32·9	9·10
A	[6-^{14}C]Glucose, 5 m*M*	0·05	80·2	10,960	59·2	9·30
A	[3-^{14}C]Pyruvate, 10 m*M* + glucose, 5 m*M*	0	67·2	5,520	12·8	2·20
A	[3-^{14}C] Pyruvate, 10 m*M* + glucose, 5 m*M*	0·05	32·5	1,520	1·3	0·20
A	None	0	3·1	—	—	—
A	None	0·05	15·7	—	—	—
B	[1,4-^{14}C]Succinate, 20 m*M*	0	6·1	278	5·4	1·94
B	[1,4-^{14}C]Succinate, 20 m*M*	0·05	10·4	45	3·0	0·73
B	[1,4-^{14}C]Succinate, 20 m*M* + glucose, 5 m*M*	0	38·2	326	10·6	1·23
B	[1,4-^{14}C]Succinate, 20 m*M* + glucose, 5 m*M*	0·05	38·2	91	5·7	0·45
B	[^{14}C]Glutamate, 20 m*M*	0	4·0	160	0·5	0·18
B	[^{14}C]Glutamate, 20 m*M*	0·05	4·5	16	0·1	0·03
B	[^{14}C]Glutamate, 20 m*M* + glucose, 5 m*M*	0	36·1	1,500	30·3	2·10
B	[^{14}C]Glutamate, 20 m*M* + glucose, 5 m*M*	0·05	40·8	250	6·8	0·50
B	Glucose, 5 m*M*	0	34·7	—	—	—
B	Glucose, 5 m*M*	0·05	42·9	—	—	—
B	None	0	4·7	—	—	—
B	None	0·05	10·0	—	—	—

Starved baker's yeast (8·1 mgm., exp. *A* and 23 mgm., exp. *B*) suspended in 2·0 ml. 25 m*M* phthalate buffer with additions as shown. 5 μc. [3-^{14}C]pyruvate and [6-^{14}C]glucose: 2·5 μc. [1,4-^{14}C]succinate and 2 μc. [^{14}C]glutamate (1 μc. $^{14}C = 1{\cdot}19\ 10^4$ counts/min.); final pH, 4·5; 0·15 ml. 5 *N* sodium hydroxide (carbonate free) in the central well of the Warburg flask; air in the gas space; temperature, 30°. After incubation (2 hr. exp. *A* and 3 hr., exp. *B*) the carbon dioxide absorbed in the alkali was precipitated as barium carbonate, washed, filtered, weighed and the radioactivity counted. The yeast suspension was diluted with 5 ml. cold phthalate buffer and centrifuged at 1°. The dilution and centrifugation were repeated and the yeast cells finally suspended in 5–10 ml. methanol for carbon-14 counting. Experimental details as before (ref. 8).

If non-radioactive glucose is added to the yeast cells in the presence of the substrates referred to and DNP, the later inhibitory action diminishes as shown by the comparatively higher incorporation of carbon-14 into the yeast cells and the total amount of carbon-14 in the carbon dioxide evolved (Table 1). Glucose effect is particularly noteworthy if it is taken into account that the carbon-14 from pyruvate becomes diluted with glucose carbon, which dilution is reflected by the relative diminution of specific activity of carbon-14 in the respiratory carbon dioxide. With the dicarboxylic acids and glutamate, the action of glucose is more striking because glucose increases absolute incorporation of carbon-14 into the yeast cells or in carbon dioxide, irrespective of the presence of DNP.

Yeast cells are divided by membrane barriers into an outer region identical with the cell wall, and a central region where organic acids like pyruvic, succinic, fumaric, glutamic, glycine, etc., do not enter or enter at a slow rate[4]. The central region includes the cytoplasm and mitochondria[5] that catalyse the citric acid cycle oxidations[6]. On these grounds, the results quoted support the hypothesis that, as in other instances[7], the transfer of substrates across the yeast membranes is an active, metabolically controlled process.

With ^{14}C-acetate as substrate, which oxidation is also inhibited by DNP[8], similar results can be observed, namely, inhibition of the oxygen uptake, the incorporation of carbon-14 into the cell constituents and the evolution of labelled carbon dioxide; the addition of glucose strongly decreases the effect of DNP. At variance with the other substrates studied, acetate diffuses freely into the yeast cell inner compartment[4], and its oxidation through the citric acid cycle in yeast requires as initial step activation with adenosine triphosphate[10], or in other words, is dependent on energy. The already established uncoupling action[6] of DNP on oxidative phosphorylation in yeast seems to be, therefore, the ultimate mechanism of the inhibition of substrate oxidation at the citric acid cycle-level. The effect of glucose can be easily understood because DNP does not inhibit phosphate esterification during glycolysis[11].

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Mechanism of Activation and Reduction of α-Aminoadipic Acid by Yeast Enzyme

Previously[1] we reported the reduction reaction of α-aminoadipic acid by a yeast enzyme(s) which will now be referred to tentatively as α-aminoadipic acid reductase. The reaction by which α-aminoadipic acid is converted to the corresponding δ-semialdehyde requires the participation of adenosine triphosphate, reduced triphosphopyridine nucleotide and Mg^{++}, and the enzyme is activated by glutathione. The present communication reports the formation of δ-adenyl-α-aminoadipic acid by the α-aminoadipic

Table 1. Requirements for the Pyrophosphate (PP)–Adenosine Triphosphate (ATP) Exchange Reaction

Complete system plus or minus	c.p.s./γp of PP in ATP	Exchange (per cent)
Complete	103	76·7
Minus α-aminoadipic acid	19	14·3
,, glutathione	115	84·9
,, Mg^{++}	46	33·7
,, Mn^{++}	106	78·3
,, Mg^{++}, Mn^{++}	45	33·1
,, α-aminoadipic acid reductase	0	0
Plus *iso*citric acid dehydrogenase*	102	76·6
Minus inorganic labelled pyrophosphate, plus labelled orthophosphate	0	0

* Purified *iso*citric acid dehydrogenase (0·5 mgm.).
Complete system consisted of (in μmoles) 2 adenosine triphosphate, 2 inorganic labelled pyrophosphate (2×10^5 c.p.m.) or ^{32}Pi (10^6 c.p.m.), 6 DL-α-aminoadipic acid, 2 magnesium chloride, 0·5 glutathione, 0·18 manganous chloride, 10 potassium fluoride, 10 barbital buffer *p*H 7·4 and 0·4 mgm. of α-aminoadipic acid reductase, in a total volume of 1·0 ml. Incubation was for 10 min. at 37°.

acid reductase, together with a possible mechanism of α-aminoadipic acid reduction reaction.

The isolation of the enzyme and steps of the purification were identical with those of the previous communication[1] except that the enzyme was dialysed for 2½ hr. against demineralized water and then for 34 hr. at 0° against 0·1 *M* potassium chloride solution containing 0·01 *M* potassium bicarbonate. After dialysis the enzyme solution was centrifuged at $10^4 g$ for 10 min. and the supernatant was saved. *Iso*citric acid dehydrogenase was purified by the method of Kornberg and Pricer[2].

The α-aminoadipic acid-dependent exchange between labelled inorganic pyrophosphate and adenosine triphosphate catalysed by the enzyme was studied by Hoagland's method[3]. The reaction was stopped with 6 per cent perchloric acid. Adenosine triphosphate was separated from the labelled pyrophosphate and determined according to the method of Craine and Lipmann[4].

Typical results are presented in Table 1. α-Aminoadipic acid reductase catalysed a rapid exchange of labelled inorganic pyrophosphate with the pyrophosphate moiety of adenosine triphosphate. The exchange reaction was dependent on the presence of α-aminoadipic acid and Mg^{++}. In contrast to the overall reduction reaction, no requirement for glutathione was observed, and monoiodoacetate also did not give any appreciable effect on the rate of the exchange reaction. This indicates that the reduction reaction of α-aminoadipic acid is separable into two coupled reactions. The exchange reaction occurred only with the intact pyrophosphate molecule without prior hydrolysis to orthophosphate. The exchange reaction was completely inhibited by versene and recovered by addition of Mg^{++}. The reaction occurred over a broad *p*H range of about 6–8 and was optimal at about *p*H 7·4. Of twelve amino-acids tested including homoglutamine, lysine, glutamic acid and aspartic acid, α-aminoadipic acid only was active in this system. It should be emphasized that homoglutamine, the δ-carboxyl group of which is blocked in the form of acid amide, was lacking in activity.

In the previous communication, we reported the formation and accumulation of a derivative of α-aminoadipic acid-δ-semialdehyde in the α-aminoadipic acid reduction system. In the subsequent experiments, it was found that under ultra-violet light the paper chromatogram of the α-aminoadipic acid-δ-semialdehyde derivative fraction showed one main spot (concomitant with other spots), the R_P (0·08–0·12, with *n*-butanol–acetic acid–water; 4·5 : 1 : 2 v/v) of which corresponds to that of the area occupied by the colour reaction with *p*-dimethylaminobenzaldehyde[1], and both are superimposable. The other spots appearing on the chromatogram may be attributed to the deteriorated products of the primary reduction product of α-aminoadipic acid, since highly labile and deterioration properties of the reduction product might be expected. When the reduction reaction was carried out in the presence of potassium fluoride (10^{-2} *M*), pyrophosphate was accumulated in the system. The reduction reaction was inhibited about 50 per cent of control by 5×10^{-4} *M* of adenosine-5′-phosphate, while pyrophosphate at higher concentrations than adenosine-5′-phosphate was only slightly inhibitory to the system, and the latter, on the other hand, had little effect on the exchange reaction.

These findings permit a tentative formulation of the process by which α-aminoadipic acid is reduced to its semialdehyde. First, α-aminoadipic acid reacts with adenosine triphosphate through the δ-carboxyl group splitting off pyrophosphate and forming possibly δ-adenyl-α-aminoadipic acid as an activated α-aminoadipic acid. δ-Adenyl-α-aminoadipic acid is then an obligatory participant in the reduction reaction, and the reactions can be written as follows:

(1) α-AAA + ATP⟶δ-adenyl-α-AAA + PP

(2) α-AAA-AMP + TPNH.H^+⟶ α-AAA-H_2-AMP + TPN^+

(3) α-AAA-H_2-AMP + H_2O⟶ α-AAA-δ-semialdehyde + AMP

These reactions would account for the strong inhibition of adenosine monophosphate to the reduction reaction of α-aminoadipic acid, as the result of adenosine monophosphate and δ-adenyl-α-aminoadipic acid competition for the enzyme at the reduction step (reaction 2). The above reactions would also account both for the α-aminoadipic acid-dependent exchange between pyrophosphate and adenosine triphosphate, and for the accumulation of a derivative of α-aminoadipic acid-δ-semialdehyde and pyrophosphate in the reduction system of α-aminoadipic acid. The primary reduction product of α-aminoadipic acid appearing in the 6 *N* hydrochloric acid eluate might be an adenosine-5′-phosphate derivative of α-aminoadipic acid-δ-semialdehyde (tentatively to be referred to as δ-adenyl-α-aminoadipic acid-δ-semialdehyde), which might equilibrate with the dehydrated form.

By assuming these structures, it is possible to account for the labile property of the primary reduction product toward heat treatment, resulting in the formation of Δ^1-piperidine-6-carboxylic acid[1].

Details of this work will be published elsewhere.

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Paper Chromatography and Paper Electrorheophoresis of Tuberculoproteins

Paper electrophoresis of proteins has on the whole proved to be a more satisfying means of separation than paper chromatography, owing to the physico-chemical properties of the proteins that make them rather unfit for the latter method[1,2]. Nevertheless there do exist methods of paper chromatographic separation of, for example, enzymes, blood and serum proteins, mainly based on a kind of adsorption analysis, in addition to the more usual procedures of partition-, ion exchange- and adsorption-chromatography on columns.

Franklin *et al.*[3] claimed to have made separations of sera into a great number of fractions by means of two-dimensional paper chromatography. Conditions and results of these procedures provoked severe criticism. Though inclined to be sceptical I, too, agreed with E. and M. Lederer[1] in their opinion on the possible value of these investigations and so undertook similar experiments. The method of two-dimensional chromatography with respectively 0·1 *M* sucrose and 0·1 *M* potassium sodium tartrate[3], applied to my bovine tuberculin purified protein derivative, and some of its fractions[4], produced strange irregular patterns of no practical value, though a certain reproducibility was observed. For practical purposes I then proceeded in the following way: 0·1 *M* sucrose in the first direction and 0·041 *M* acetic acid + 0·009 *M* sodium acetate in the second. The *p*H of this last-named buffer, being 4, is the isoelectric precipitation point of the *C* fraction under certain defined conditions[5].

Fig. 1 shows that with my chromatography these conditions were rather well fulfilled.

The corresponding preparations from non-heated culture filtrates[5] gave the same pictures; only the ratio $A+B/C$ was higher[6] as expected and demonstrated by quantitative colorimetric measurement of the eluates. The patterns are in their entirety reproducible, but not in detail. So, for example, the $A+B$ spots vary in shape and show a division into two or more parts. I doubt if there can be attributed any meaning to this partition from the viewpoint of protein separation. How far we are dealing with artefacts remains to be investigated. My method, based on a previously known chemical separation and therefore somewhat dissatisfying like others of this kind[7], can be used in comparing different tuberculin samples.

Among many other experiments only chromatography with pyridine/sucrose solutions, combined with electrophoresis in borate buffers, showed patterns of some reproducibility, though as yet not good enough for practical use.

The difficulties encountered in paper electrophoresis of heated tuberculoproteins, the properties of which are apparently unfavourable for this kind of separation, have been mentioned before[4]. In this case the molecular weight of the *C* protein is twice the normal, while the *A* and *B* fractions consist probably of molecules smaller than when they do not originate from heat-sterilized culture media[8], facts that may account for the greater separation troubles[9]. In trying to cancel unfavourable effects in paper

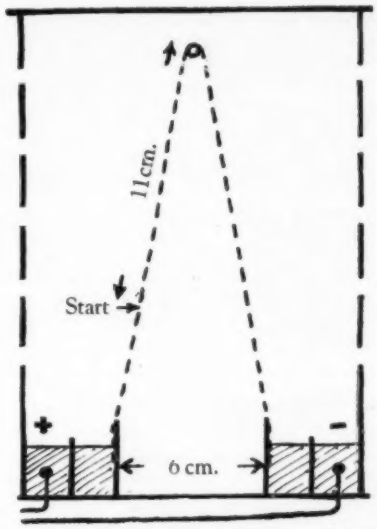

Fig. 2. Cross-section through a 'Perspex' electrophoresis apparatus of the Macheboeuf type

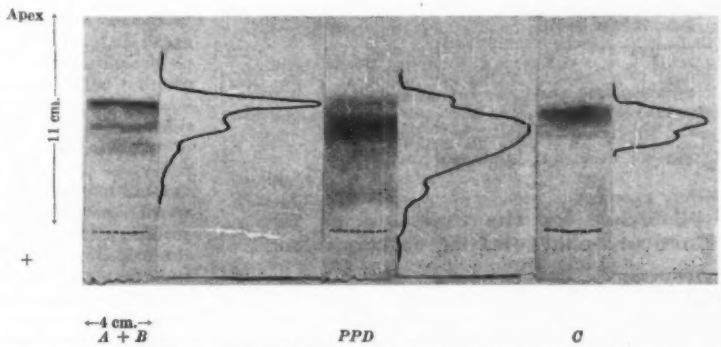

Fig. 3. Electrorheophoresis of 0·05 ml. protein samples (50 mgm./ml.). The curves were obtained with a Zeiss scanner

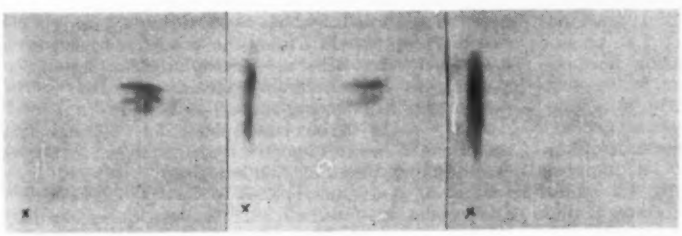

Fig. 1. Two-dimensional chromatography at 4° C. for about 3 hr. in each direction. Circular spots of 0·02 ml. protein solution of about 50 mgm./ml. In between, drying in the cold, for example, with a fan. Staining of the heat-dried Whatman No. 1 sheets with 'Amidoschwarz 10*B*' (Bayer). Dimensions of the sheets, 11 in. × 11 in.

electrophoresis due to the above-mentioned protein properties, I found a method, based on the electrorheophoresis principle of Macheboeuf[10], in which a rather vigorous evaporation-flow counteracts the electrophoretic movement of the proteins. The apparatus and results are shown in Figs. 2 and 3. A *p*H 8 buffer was used, consisting of 10 gm./l. boric acid, 0·5 gm./l. disodium salt of ethylene di-amino-tetraacetic acid, 0·05 ml./l. of 'Tween 80' and *tris* hydroxy methyl amino-methane so as to make the *p*H of the solution 8. Separations took place on Schleicher and Schüll paper No. 4032*b Mgl* at 7–9 V./cm. during 4–6 hr. at 24° C. For standardizing electrophoresis conditions and identification of the fractions obtained further investigations remain to be carried out.

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Evidence for the Existence of Two Corticotrophin-releasing Factors, α and β

We wish to report here the presence of two distinct peptides with corticotrophin-releasing factor activity in hog posterior pituitary extracts. All the separation methods as well as the bioassays for corticotrophin-releasing factor, adrenocorticotrophic hormone, vasopressin and melanocyte stimulating hormone have been described previously[1–6].

A single peak of basic peptides obtained by preparative chromatography on carboxymethylcellulose had as its main components lysine-vasopressin and α-melanocyte stimulating hormone[3]. This peak, which also contained all the corticotrophin-releasing factor activity, was distributed for 100 transfers in a system of *n*-butanol/0·03 *M* *p*-toluene sulphonic acid. Lysine-vasopressin ($K = 0{\cdot}32$, 300 pressor u./mgm.) was well separated from the α-melanocyte stimulating hormone area ($K = 6$, $1{\cdot}3 \times 10^7$ melanocyte stimulating hormone u./mgm., 0·2 pressor u./mgm.). The α-melanocyte stimulating hormone area contained corticotrophin-releasing factor activity, which could be separated from the α-melanocyte stimulating hormone on analytical scale carboxymethylcellulose columns. This corticotrophin-releasing factor activity was found to be due to a peptide or a group of peptides containing the amino-acids of α-melanocyte stimulating hormone[7] plus threonine, alanine and leucine. The peptide with corticotrophin-releasing factor activity apparently related to α-melanocyte stimulating hormone, containing methionine as the sulphur amino-acid, has been designated α-corticotrophin-releasing factor.

Some inherent adrenocorticotrophic hormone activity of α-corticotrophin-releasing factor (<0·2 u./mgm.) and the presence of alanine and leucine support the concept that this molecule may serve as a biosynthetic precursor of adrenocorticotrophic hormone. The peptide obtained by Gros and Privat de Garilhe[8] appears to be similar or identical to α-corticotrophin-releasing factor. Since the latter was active in releasing adrenocorticotrophic hormone only at relatively high levels of 2–3 μgm. *in vivo* or 0·5γ *in vitro*, this discrepancy in corticotrophin-releasing factor activities as compared with our previous results[2–4,6] had to be explained. Similarly the amino-acid composition of α-corticotrophin-releasing factor closely resembling that of α-melanocyte stimulating hormone led us to believe that we were dealing, in α-corticotrophin-releasing factor, with a substance quite different from the one we had purified earlier[1–4], which appeared to be related to vasopressin.

The peak of pressor activity in the countercurrent distribution system was then carefully explored. It was possible to detect corticotrophin-releasing factor activity on the descending edge of the vasopressin peak after countercurrent distribution. A separation from vasopressin was achieved by paper partition chromatography[3]. Corticotrophin-releasing factor activity was found to be due to a small peptide, apparently related to vasopressin, on the basis of preliminary amino-acid composition and chromatographic behaviour. This peptide, active at 0·05γ *in vitro* or 0·1γ *in vivo*, was designated β-corticotrophin-releasing factor. Additional support for the concept of two distinct corticotrophin-releasing factor molecules was provided by the detection of two corticotrophin releasing factor active areas with different specific activities on an analytical scale column of carboxymethylcellulose. This was achieved under conditions in which the order of elution of vasopressin and α-melanocyte stimulating hormone is reversed[5], the starting material being posterior pituitary concentrates[3].

Since both α- and β-corticotrophin-releasing factor are very basic peptides, our results are not in agreement with the data of Royce and Sayers[9]. One of the two corticotrophin-releasing factor active areas obtained by these authors after chromatography of median eminence extracts on carboxymethylcellulose must be explained by the presence of an acidic component or more probably, according to our own results, by overloading of the column. In our work, results from preparative chromatography of median eminence extracts on carboxymethylcellulose have revealed the presence of only one corticotrophin-releasing factor active area, in the position occupied by basic peptides emerging just before vasopressin. Under the conditions used here it is likely that this corticotrophin-releasing factor activity in hypothalamic extracts was due to β-corticotrophin-releasing factor, although on a preparative scale carboxymethylcellulose column the presence of some α-corticotrophin-releasing factor cannot be eliminated with certainty.

In view of the discrepancy in the specific activities and preliminary amino-acid composition of the two substances, one might propose that β-corticotrophin-releasing factor is the true chemical mediator for release of adrenocorticotrophic hormone, whereas α-corticotrophin-releasing factor is an α-melanocyte stimulating hormone analogue, possibly a pre-

cursor in the biosynthesis of adrenocorticotrophic hormone.

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Spontaneous Plasma Kinin Formation in Human Plasma collected during Labour

Deficiency of substrate for induced plasma kinin production in the stored plasmas collected from women at the time of delivery has been shown[1]. This substrate deficiency was not observed in normal plasma (that is, from non-parturient individuals) stored for long periods at −20° C., or in plasma collected at time of delivery from women undergoing Cæsarean section at term before the onset of labour.

In the present series of experiments the plasmas were collected as previously described[1]; but, as soon as possible after their collection (5–25 min.), plasma aliquots were placed in polythene containers at 0° C. and tested forthwith, in doses of 0·1–0·01 ml., on the isolated rat uterus in a 10 ml. bath. It was then found that plasmas collected from women in labour showed marked oxytocic activity, which increased further for ½–1½ hr. This unusual activity was observed a short time before the onset of labour. The rapidity of its development increased throughout the first and second stages reaching, at its maximum, an activity equivalent to several units of oxytocin ('Syntocinon', Sandoz) per ml. plasma. It then declined, but was demonstrable for a variable period (usually 1–4 hr.) after completion of the third stage. These findings are in contradistinction to the negligible oxytocic activity which develops in normal plasma kept in polythene containers at 0° C. for 1 hr.[2].

The spontaneously produced oxytocic activity in parturition plasmas kept at 0° C. resembled that due to plasma kinin[3]. It rapidly disappeared when the temperature of the plasma was raised to 20° C. Conversely, the rate of disappearance of kinin added to plasma was markedly reduced when the plasma temperature was lowered from 20° C. to 0° C. This can be explained by the influence of temperature on plasma kininase. Since we have found the same kininase activity in normal and parturition plasmas, we consider that the rapidly developing oxytocic power of the latter, at 0° C., was almost certainly due to abnormally rapid formation.

The amount of spontaneous kinin formation in plasma samples collected at different stages of parturition was directly correlated with the degree of substrate depletion measured at these different stages[1].

We think it unlikely that plasmin was the enzyme responsible for the spontaneous production of oxytocic activity since the peak period for development of the latter in the plasma, and the subsequent depletion of kininogen, corresponds with the nadir of fibrinolytic activity reported in pregnancy plasma[4]. Moreover, normal plasma with high fibrinolytic activity (for example, after severe exercise or surgical operations) failed to develop a similar marked oxytocic activity. In addition, agents such as heparin (25 I.U./ml.) and ε-amino caproic acid (0·12 *M*), which inhibit plasmin, promoted the spontaneous kinin formation. These substances also potentiate kinin formation in normal plasma when it is exposed to glass, and this is known to be independent of the plasminogen–plasmin system[5,6].

The active oxytocic principle did not develop in parturition plasma to which the soya bean trypsin inhibitor (2 mgm./ml.) had been added. Like plasma kinin, it was extractable in boiling ethanol from active (that is, oxytocic) plasma kept at 0° C., but not from control samples of the same plasma kept at room temperature, and the activity of the extracts was rapidly destroyed by a crude amino-peptidase (*E. coli*) preparation. It is our opinion that it is probably a polypeptide.

The oxytocic activity in plasma or extract, unlike oxytocin, was not rapidly destroyed by sodium thioglycollate, though the build-up of kinin in parturition plasma kept at 0° C. was inhibited by this agent. In these respects the spontaneous kinin production resembled that of kinin formed by exposure of human plasma to glass, and, to a certain extent, the formation of bradykinin by trypsin added to human pre-active plasma. Differences in dose–response relationships between the spontaneously arising kinin of parturition plasma and oxytocin, and the marked lack of tachyphylactic response of the uterus towards the former, also suggest that we were not dealing with oxytocin itself.

The increasing rapidity of development of the parturition kinin throughout labour, reaching its maximum at the end of the second stage, suggests that the oxytocic activity may be related to the increasingly powerful contractions observed in the human uterus up to this time. The source of the active enzyme producing this oxytocic activity is not at present known.

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Isolation of Hyaluronate from Human Synovial Fluid by Zone Electrophoresis

Little is known about the condition of hyaluronate in normal human synovial fluid because it is difficult to isolate the former in good yield by mild methods from small quantities of highly viscous fluid. Although hyaluronate can be completely precipitated from small amounts of synovial fluid by adding acetic acid or a complex cobalt salt, some degradation of hyaluronate may occur, and 50–60 per cent of the proteins of the fluid are co-precipitated in the mucin clot[1]. Isolation of hyaluronate from synovial fluid by moving-boundary electrophoresis is feasible, for at *p*H 8·6 hyaluronate migrates toward the anode slightly ahead of albumin. In normal synovial fluid, one or two components that migrated ahead of albumin were found[2] but were not isolated for further study. Electrodeposition of pooled synovial fluids was carried out three times at *p*H 7·0 by Roseman *et al.*[3], and hyaluronate was isolated in yields of 70 per cent or less, with a ratio mgm. protein/mgm. hyaluronate of 0·13, or about 11·5 per cent protein in the hyaluronate.

In the present work the use of zone electrophoresis permitted hyaluronate to be isolated from small quantities of viscous fluid and recovered for analysis. A weighed amount (about 1 gm.) of synovial fluid from apparently normal knee joints of deceased or living subjects was diluted to 5 gm. with a phosphate buffer (0·047 *M*, *p*H 6·5) and then dialysed for about 18 hr. at 2° C. against 500 ml. of an acetate buffer (0·087 *M*, $\Gamma/2 = 0{\cdot}075$, *p*H 5·4) changed twice (10·2 gm. crystalline sodium acetate and 0·7 ml. glacial acetic acid diluted to 1 litre with distilled water that was also de-ionized). An aliquot (about 0·1 gm.) of the diluted and dialysed synovial fluid was analysed for hexuronic acid[4]. The protein content of diluted and dialysed normal synovial fluid is too low to interfere with this analysis. All the hexuronic acid measured is a component of hyaluronate, since testicular hyaluronidase hydrolyses and renders dialysable 95 per cent of the hexuronic acid of the fluid.

The remainder of the diluted and dialysed synovial fluid was applied to a 2½-in. long slit in a solid block (18 in. × 5 in. × ½ in.) of polyvinyl chloride ('Geon' resin No. 427 from B. F. Goodrich Chemical Co., Cleveland, Ohio). Electrophoresis was carried out in the acetate buffer at 2° C. either for 14 hr. at a voltage gradient of 8·7 V./cm., or for 21¼ hr. at a gradient of 5·5 V./cm. The equipment and technique were modified from that used for zone electrophoresis of serum[5].

After electrophoresis, strips of Whatman No. 1 filter paper were pressed on the wet block and were dried at 90° C. for 20 min. The paper strips were stained with bromphenol blue or toluidine blue to locate approximately the positions on the block of proteins and hyaluronate respectively. The block was cut into segments (½ in. wide × 3½ in. long) starting near the anodal end. Each segment was placed in a sintered glass (coarse) funnel and eluted with a total of 10 ml. of a buffer (0·01 *M* sodium bicarbonate, 0·05 *M* sodium chloride, *p*H 8·1) by applying air pressure to the funnel. The eluates were evaporated to dryness *in vacuo* and then dissolved in 2 ml. of de-ionized distilled water. Insoluble resin was removed by centrifugation. All the eluates were examined for protein by a modified Folin method. Each eluate containing hyaluronate was analysed for hexuronic acid. (Hexuronic acid × 2·5 = hyaluronate.)

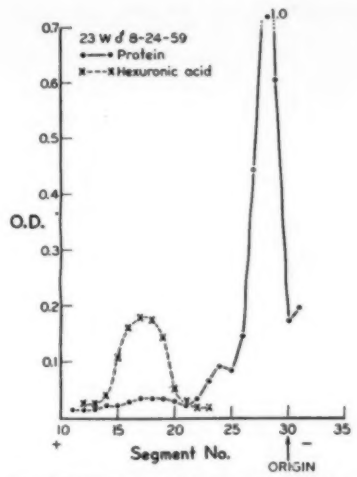

Fig. 1. Electrophoresis of normal human synovial fluid in acetate buffer (*p*H 5·4) on a block of polyvinyl chloride for 20¼ hr. at a gradient of 5·5 V./cm. Hyaluronate zone, segments 14–20; orosomucoid, segments 22–25; albumin forms the main component of segments 26–28

At *p*H 5·4 hyaluronate migrated faster than most of the proteins of synovial fluid, but a small amount of protein was present in the hyaluronate zone (Fig. 1). Hyaluronate was recovered in a yield of 80–90 per cent (Table 1). Further results shown in Table 1 were obtained by pooling all the eluates containing hyaluronate and measuring hexuronic acid and protein (Red Cross Fraction 2 was used as the protein standard). These experiments were done only when the zone of hyaluronate and protein was completely separated from the rest of the proteins of synovial fluid, as shown in Fig. 1. The ratio mgm.

Table 1. Analyses of the Hyaluronate Zone after Electrophoresis on Blocks of Polyvinyl Chloride of Normal Human Synovial Fluids in Acetate Buffer *p*H 5·4

Synovial fluid*	Voltage gradient (V./cm.)	Hyaluronate: Applied (mgm.)	Hyaluronate: Recovered (mgm.)	Hyaluronate: Recovered (per cent)	Pooled eluates, hyaluronate zone: Protein (mgm./gm.)	Pooled eluates, hyaluronate zone: Hyaluronate (mgm./gm.)	Pooled eluates, hyaluronate zone: Ratio (mgm. protein/mgm. hyaluronate)
23†	5·5	3·45	2·83	82	0·192	1·90	0·10
33	8·7	5·15	4·90	95	0·096	1·54	0·06
38	8·7	5·18	3·78	73	0·052	1·62	0·03
41	8·7	4·39	3·70	84	0·031	0·97	0·03
59	8·7	3·73	3·18	86	0·022	0·91	0·02
62	8·7	2·75	2·57	94	0·152	1·29	0·12
70	5·5	3·10	2·78	90	0·107	1·31	0·08

* Age of subjects from whom fluid was obtained.
† Part of this synovial fluid was also incubated with testicular hyaluronidase and zone electrophoresis was then carried out. Hyaluronate fragments were recovered in a yield of 80 per cent and the ratio mgm. protein/mgm. hyaluronate fragments was 0·018.

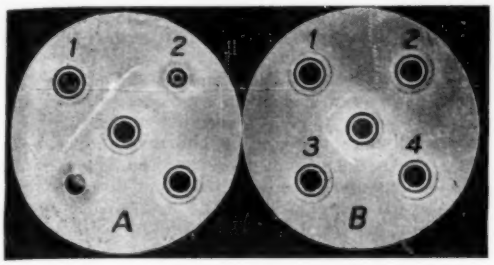

Fig. 2. Double gel diffusion in agar of pooled, concentrated eluates from normal synovial fluid after zone electrophoresis. *A*, Centre cup, anti-γ-globulin (absorbed with fraction V and a β-globulin fraction). 1, Fraction II, 0·2 per cent. 2, Hyaluronate zone (segments 14–20) from normal synovial fluid shown in Fig. 1. A faint, broad precipitin line formed near slowly diffusing antigen. Concentration of antibody was in excess. *B*, Centre cup, anti-fraction VI. Orosomucoid standard 0·025 per cent. 2, Fastest migrating protein component of normal serum isolated by zone electrophoresis at *p*H 5·4. 3, Protein (segments 22–25) from normal synovial fluid shown in Fig. 1. 4, Protein that migrated just behind hyaluronate zone isolated by zone electrophoresis of another normal synovial fluid. Precipitin lines formed by all antigens coalesced (reaction of identity). These experiments were carried out by Dr. L. Korngold

protein/mgm. hyaluronate of the isolated hyaluronate was 0·02–0·12. Double-diffusion studies in agar showed that the protein in two samples (Table 1, 23 and 62) of isolated hyaluronate formed a precipitin line against anti-γ-globulin (Fig. 2). Neither orosomucoid nor albumin, which migrated just behind the hyaluronate zone, could be detected in these samples of hyaluronate by this immunological method.

Eluates containing the isolated hyaluronate from four synovial fluids (Table 1, 33, 38, 41, 59) were pooled, concentrated to a small volume and dialysed in distilled water to remove salts. After adding potassium acetate (1 per cent w/v) and 1 vol. of cold ethanol, part of the hyaluronate formed a ropy clot. Addition of two more volumes of ethanol resulted in a flocculent precipitate of more hyaluronate. Each precipitate was separately washed with ethanol and ether, and dried over phosphorus pentoxide in a vacuum. Analytical results are shown in Table 2. The hyaluronate obtained contained the lowest quantity of protein (4 per cent) yet reported when mild methods have been used for isolation. The calculated protein content of the hyaluronate obtained by three electrodepositions[3] was 11·5 per cent; the isolation of protein-free hyaluronate was reported in a later abstract[6], but analyses were not given. Ogston and Stanier, after 16 or more ultrafiltrations of bovine synovial fluid, isolated hyaluronate in the ultra-filter residue in what was called a firm complex with about 25 per cent protein, composed largely of α-globulins, with smaller amounts of β- and γ-globulins[7]. Ogston and Sherman recently found[8] that 65 per cent of the protein in the complex could be removed by proteolytic enzymes without altering the physical properties of the hyaluronate in solution. In the present work the finding of γ-globulin in the hyaluronate zone is unexpected and worth further study.

Table 2. Analyses of Hyaluronate obtained by Zone Electrophoresis of Normal Human Synovial Fluids

Eluates containing the isolated hyaluronate from four synovial fluids were pooled. On addition of 1 vol. of ethanol part of the hyaluronate was precipitated as a ropy clot; addition of two more volumes of ethanol caused a flocculent precipitate of hyaluronate. Each precipitate was separately dried and analysed

	Ethanol added 1 vol.	Ethanol added 3 vol.
Yield (mgm.)	2·8	1·3
Hexosamine (per cent)	28·6	
Hexuronic acid (per cent)	31·6	34·2
Molar ratio $\frac{\text{hexosamine}}{\text{hexuronic acid}}$	0·98	
Nitrogen (per cent)	2·88	3·36
Molar ratio $\frac{\text{nitrogen}}{\text{hexosamine}}$	1·26	1·36
Protein (per cent)	2·47	4·53

This work was supported by grant 2*A*-5082 from the National Institute of Arthritis and Metabolic Diseases, and by the New York State Chapter, Arthritis and Rheumatism Foundation. We are grateful to Drs. H. Kunkel and E. Franklin for advice concerning zone electrophoresis; and to Dr. M. Schubert for helpful discussions.

Note added in proof. Further to identify protein in the isolated hyaluronate, rabbits were injected with this material in Freund's adjuvant. Antibodies to α_2-globulin as well as to γ-globulin were detected by the tanned cell hæmagglutination technique.

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Inhibitor of the Cytochrome Oxidase of Unfertilized Sea Urchin Eggs

In a previous communication[1] the presence of an inhibitor of cytochrome oxidase in the unfertilized sea urchin egg was described. It was shown that its activity decreases considerably following fertilization, that it is a low-molecular compound, that it is heat- and alkali-labile and that its action is of a competitive type.

We wish to report here some further experiments aimed mainly at obtaining the inhibitor in a purified form and at finding out about its chemical nature and its mode of action.

Fig. 1 illustrates a typical experiment showing that the inhibitor (prepared as described by Maggio and Monroy[1]) interferes with the oxidation of reduced cytochrome *c* by cytochrome oxidase (a preparation of mitochondrial membranes[2]). On the other hand, we have found that the reduction of cytochrome *c* is not inhibited. These results, taken together with the previous ones on the competitive type of the inhibition, suggest that the mechanism of the inhibition may be interference with the oxidation of cytochrome oxidase.

Most of our efforts have been directed towards the purification of the inhibitory substance. After several unsuccessful attempts, encouraging results have been obtained using columns of 'Ecteola' cellulose. Homogenates of jelly-free unfertilized eggs of *Paracentrotus lividus* in 0·05 *M* phosphate buffer at *p*H 7·2 were centrifuged at 12,000*g* for 15 min. in the cold and the supernatant centrifuged again at 105,000*g* for 1 hr. The final supernatant was dialysed overnight against distilled water and the dialysate

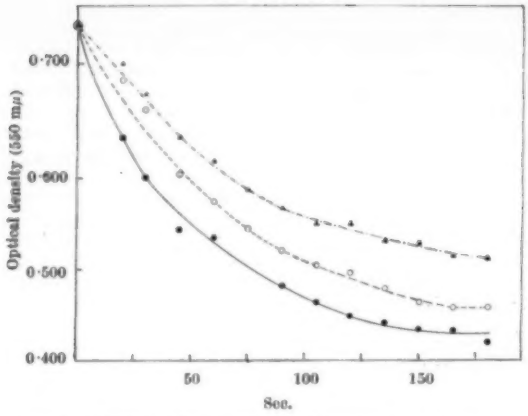

Fig. 1. Inhibition of the re-oxidation of cytochrome *c* by a crude preparation of the inhibitor (effluent from a 'Dowex-50' column, see ref. 1). The system contained in the final volume of 3·3 ml. of 0·1 *M* phosphate buffer *p*H 7·4, $2{\cdot}78 \times 10^{-5}$ *M* cytochrome *c*, $8{\cdot}1 \times 10^{-5}$ *M* sodium ascorbate, mitochondrial membranes from rat liver (0·1 ml. containing 0·1 mgm. protein). ●, control; ○, + 0·3 ml. of inhibitor; ▲, + 0·6 ml. of inhibitor. The re-oxidation of cytochrome *c* is indicated by the decrease of the absorbance at 550 mμ

containing the inhibitor was lyophilized. The dry material was dissolved in cold glass-distilled water to a final concentration of 0·04 *M* phosphate and the *p*H adjusted to 7·2. This solution was passed through an 'Ecteola' column prepared in phosphate buffer 0·04 *M*, *p*H 7·2. The unadsorbed material was collected, together with the first washing (carried out with 20 ml. of phosphate 0·04 *M*, *p*H 7·2), which was followed by a stepwise elution with 10 ml. portions of 0·04 *M* phosphate at *p*H 6·5, 6·0 and 5·0. The washing and the eluates were lyophilized, redissolved in distilled water to a final concentration of 0·08 *M*, and, after adjusting the *p*H to 7·2, assayed for the cytochrome oxidase inhibiting activity by the manometric technique, as already described[1]. The results of two such experiments are given in Table 1. While in most cases the activity was present only in the *p*H 5·0 fraction, in others, as in Exp. 2, some activity was eluted also with the other fractions. We take this as an indication that our method of fractionation is not yet entirely satisfactory. No ninhydrin-positive spots appeared in the chromatograms of the *p*H 5·0 fraction and nitrogen determinations were also negative. Hence it may be concluded that the inhibitor is not a nitrogen-containing substance.

It has happened sometimes, especially during purification, either that an originally active preparation lost its activity in the course of purification, or that the preparations were inactive from the beginning. We have then tried to re-activate such inactive inhibitors and positive results have been obtained with reduced glutathione and cysteine. The former compound has given more constant results, evidently due to its greater stability at the neutral or slightly alkaline *p*H used in the manometric tests. When the re-activation was tried on the fractions obtained from the column, this was obtained only with the *p*H 5·0 fraction. The results of a typical experiment are illustrated in Table 2.

Table 1. Cytochrome Oxidase Inhibiting Activity of the Fractions obtained from an 'Ecteola' Column

	Exp. 1		Exp. 2	
	Q_{O_2}	Δ (per cent)	Q_{O_2}	Δ (per cent)
Control	441		404	
Washing			380	−5·95
Eluate *p*H 6·5			371	−8·20
Eluate *p*H 6·0	479	+ 8·6	320	−20·8
Eluate *p*H 5·0	284	−35·6	274	−32·2

Each Warburg vessel contained, in the final volume of 3·0 ml., 0·2 ml. of rat liver mitochondria, 0·2 ml. of cytochrome *c* $2{\cdot}46 \times 10^{-4}$ *M*, sodium ascorbate 30 μ*M*, phosphate buffer 0·048 *M*, *p*H 7·4, sample of the inhibitor 0·6 ml. Values are expressed as μl. oxygen/hr./mgm. of mitochondrial proteins (Q_{O_2}).

Table 2. Re-activation by Reduced Glutathione 10^{-3} *M* Final Concentration. Other Conditions as in Table 1

	Q_O	Δ (per cent)
Control mitochondria	548	
Mitochondria + glutathione	576	+ 5·1
Mitochondria + *p*H 5·0 eluate	590	+ 7·2
Mitochondria + *p*H 5·0 eluate + glutathione	474	−13·5

These results seem to suggest that thiol groups may be responsible for the inhibitory activity.

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PHYSIOLOGY

Thyroid Function and Oxidative Phosphorylation in Cold-exposed Rats

The presence of the thyroid gland appears to be necessary for prolonged survival of the cold-acclimatized rat[1,2]. Rats in the cold also appear to turn over iodine-131 more rapidly than normal[3–6], indicating a more rapid than normal accumulation of iodide and release of thyroid hormone. Since oxidative phosphorylation, a process thought by some[7–10] to be regulated by the thyroid, has been noted to be partially uncoupled[11–13], as well as physically and biochemically more labile in the cold-acclimatized rat[12,14], we have investigated the alteration of mitochondrial function following thyroidectomy of cold-exposed and normal rats.

Rats used in these experiments were exposed to 0–2° C. for 4–5 weeks. Thyroids were removed surgically at this time, and three weeks were allowed for recovery. The completeness of thyroidectomy was ascertained by relative concentrations of labelled potassium iodide in the neck region versus leg musculature 5–7 days after isotope injection. Ratios between 1 and 2 were accepted as indicating complete thyroidectomy. Controls were maintained at 25° C. Twice-washed liver mitochondria were prepared essentially according to Schneider and Hogeboom[15] in 0·25 *M* sucrose. Oxidation was measured by Warburg manometry and phosphate esterification according to the isotope distribution method of Lindberg and Ernster[16]. The lability of the mitochondrial oxida-

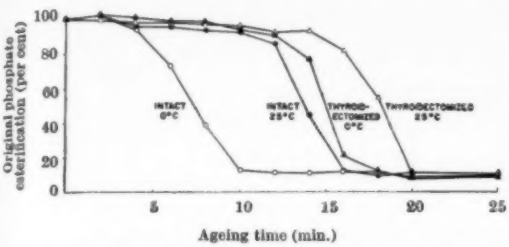

Fig. 1. Loss of phosphorylative ability of mitochondria during ageing

tive phosphorylation system was measured according to Ernster and Löw[17]. Lability of phosphorylative ability is expressed as the 'Ageing Index^{P}', which is equal to the time of ageing, in minutes, required to depress the phosphorylative capacity to a value halfway between maximal and minimal phosphorylation. Values of the statistical quantity t were obtained by a small sample, paired comparison method[18], and P derived from standard probability tables.

No difference was observed in the efficiency of oxidative phosphorylation (P/O) between preparations from control and thyroidectomized rats maintained at 25° C. (Table 1). On the other hand, the same mitochondrial suspensions showed a greater resistance to ageing on the part of the thyroidectomized preparation, this phenomenon being reflected in the difference in 'Ageing Index^{P}'. A comparison of the P/O ratios of mitochondria from intact animals, one group maintained for 4–5 weeks at 0–2° C., the other at 25° C., confirms previously published data on the lowering of the phosphorylative efficiency[11] and the 'Ageing Index^{12}' of the cold-exposed group. Thyroidectomy of the cold-exposed rat results in mitochondrial preparations with normal phosphorylative efficiency as well as a normal 'Ageing Index^{P}'. When preparations from rats maintained at 25° C. and 0–2° C., and which have been thyroidectomized are compared, the P/O ratios are normal while the 'Ageing Index^{P}' of the 25° C. thyroidectomized preparation is greatly increased. Ageing curves from a representative experiment involving the four preparations discussed above are presented in Fig. 1. The 'Ageing Indices^{P}' were calculated from such data.

These data indicate that: (1) The stability of the oxidative phosphorylation system may be in part regulated by thyroid hormone. (2) Under conditions where the P/O ratio does not reflect thyroid regulation (hypothyroidism) an indication of regulation may be observed by the determination of the 'Ageing Index^{P}'. (3) The rat is not wholly dependent on an intact thyroid to regulate uncoupling of oxidative phosphorylation and 'Ageing Index^{P}' since the P/O ratio and 'Ageing Index^{P}' of thyroidectomized preparations are returned to normal in the cold-exposed animal by thyroidectomy, but not to the cold-exposed level, indicating a control in addition to the thyroid state. This type of control may reside in the tissue itself and may consist of an autoregulatory device on the tissue-level of organization.

Table 1. The Effect of Thyroidectomy on the P/O Ratio and the Response to Ageing of Liver Mitochondria from Control and 4–5-week Cold-exposed Rats

No. of exp.	Environmental temperature	P/O		P	'Ageing index^{P}'		P
		Intact	Athyroid		Intact	Athyroid	
7	25° C.	1·84	1·82	>0·57	14·2	>21·4	0·02
8	25° C.	1·83		0·03	14·0		<0·01
	0–2° C.	1·48			8·3		
8	25° C.	1·83		0·54			
	0–2° C.		1·80		14·0	13·0	<0·57
7	25° C.		1·84	0·51		>21·4	0·01
	0–2° C.		1·80			13·6	

Each Warburg or Erlenmeyer flask contained: Mitochondria from 1/20th rat liver, 50 μmoles dipotassium hydrogen phosphate; 150 μmoles potassium chloride; 4·3 μmoles adenylic acid; 60 μmoles glucose; 125 μmoles sucrose; 30 μmoles succinate; 7·5 μmoles magnesium sulphate; and 330 *KM* units hexokinase; final *p*H, 7·4. 20 ml. reaction volume, 20 min. incubation, air gas phase. Oxygen consumption extrapolated for initial 5 min. (ref. 17).

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Abolition by 2 : 4-Dinitrophenol of the Protective Effect of Succinate and Adenine Nucleotides on the Onset of the Thyroxine-induced Swelling of Liver Mitochondria

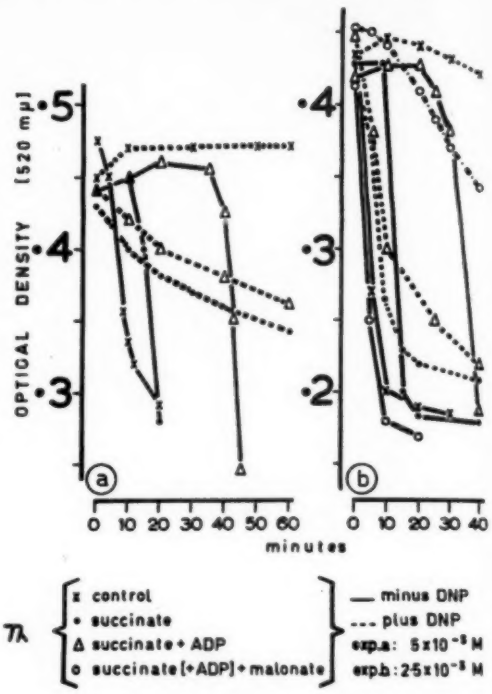
Thyroxine is known[1,2] to induce a rapid and extensive swelling of freshly isolated rat and mouse liver mitochondria suspended in a deficient medium. It has been reported recently[3] that succinate (5×10^{-4} *M*) and adenine nucleotides (10^{-5} *M*) protect against the onset of this swelling for a certain time, but not against the extent of swelling ultimately reached. The former effect was very pronounced with liver mitochondria from rats maintained on a polished-rice diet for 3–12 months. These experiments were carried out with inbred albino rats of the strain *R*-Amsterdam and F_2(*C57* Black × *CBA*) hybrid mice. It has now been found that the protective effect is weaker when liver mitochondria from several inbred strains of mice (*CBA*, *C57* Black and O_{20}-Amsterdam) or from a partly inbred strain

(*LE*) of piebald rats are used than with the particles isolated from the livers of the former animals, all animals being maintained on the standard laboratory diet. The order of activity of the nucleotides appeared to be: adenosine di- > -tri- >> or > -monophosphate, the latter being in many cases scarcely effective, if at all. Evidence has been obtained[8] that the adenine nucleotides, added in a sufficiently low concentration as to make any chelating effect unlikely, were especially active when added in conjunction with succinate. The conclusion was, therefore, drawn that the protective effect of the latter compounds on the onset of the thyroxine-induced swelling (that is, the retardation thereof) was mediated by promoting the process of oxidative phosphorylation under the adverse conditions of the deficient medium. This view has now been substantiated by the following results, which may also shed some further light on the anomalous effects of 2 : 4-dinitrophenol (DNP) on the swelling of isolated liver mitochondria.

DNP has been shown[1,2] to abolish completely the spontaneous or thyroxine-induced swelling of liver mitochondria suspended in deficient medium, but to induce swelling when oxidizable substrate is also present[4–6]. When taking into account that electron transport in the respiratory chain is required for swelling[6–8] and that DNP may inhibit respiration[3], the conclusion can be drawn (compare ref. 6) that DNP abolishes the spontaneous or thyroxine-induced swelling by inhibiting the electron transport from intramitochondrial substrates, and thus prevents swelling. Since there is also evidence[3] that added oxidizable substrates may counteract the inhibitory effect of DNP on respiration, the above anomaly may be more apparent than real. In this connexion it should be noted, when comparing the concentrations of DNP used in manometric and swelling experiments, that the mitochondria in the latter case are much more diluted than in the former.

Figs. 1*a* and *b* represent two experiments in which effects of DNP ($5 \times$ and $2{\cdot}5 \times 10^{-5}$ *M*, respectively) on the pattern of swelling of *R*-rat liver mitochondria in the presence of thyroxine, succinate and adenosine diphosphate (ADP) were studied, using the same technique as described previously[9]. In both cases DNP not only inhibited the thyroxine-induced swelling of the particles observed in the absence of added substrate, but also abolished the protective effects of succinate and succinate plus ADP on the onset of the thyroxine-induced swelling. In the first experiment with 5×10^{-5}, but not in the second with $2{\cdot}5 \times 10^{-5}$ *M* DNP, the rate of swelling in the presence of thyroxine (3×10^{-5} *M*), DNP and succinate (and ADP) was much less pronounced than that observed in the absence of DNP once the protective effect of succinate (and ADP) was overcome. This difference may be accounted for by assuming that 5×10^{-5} *M*, in contrast to $2{\cdot}5 \times 10^{-5}$ *M* DNP, still inhibited the electron transport from succinate to a certain extent. If so, it might be expected that, when the oxidation of the latter is prevented by malonate (5×10^{-3} *M*), the inhibitory effect of DNP on the electron transport, now stemming mainly from the endogenous substrates, may predominate again and thus abolish the swelling effect of thyroxine. Fig. 1*b* shows the latter prediction to be true for the low concentration of DNP.

The finding that the protective effect of succinate and adenine nucleotide on the onset of the thyroxine-induced swelling is abolished by either malonate or



Figs. 1, *a* and *b*. Effect of 2 : 4-dinitrophenol and/or malonate on the thyroxine-induced swelling of fresh rat liver mitochondria incubated in the presence of succinate (and adenosine diphosphate). 2 : 4-Dinitrophenol, 5×10^{-5} *M* in exp. *a* and $2{\cdot}5 \times 10^{-5}$ *M* in exp. *b*; DL-thyroxine, 3×10^{-5} *M*, present in each flask; succinate, 5×10^{-4} *M*; adenosine diphosphate, $1{\cdot}5 \times 10^{-5}$ *M*; malonate, 5×10^{-3} *M*. Note that the curves indicated by small circles in Fig. 1*b* represent the situation as found in the absence and presence of adenosine diphosphate

DNP indicates that the former effect is dependent on both the oxidation of succinate and the accompanying phosphorylation.

The *in vitro* swelling action of thyroxine on liver mitochondria has been shown[10] to represent an acceleration of a process which the mitochondria are already inherently capable of undergoing spontaneously; a similar conclusion has been drawn previously[11] in regard to the loss of diphosphopyridine nucleotide from isolated mitochondria, the action of thyroxine being then described as representing an acceleration of the structural and biochemical labilization of the particles such as is manifested after their ageing. The correspondence between spontaneous and thyroxine-induced swelling was further substantiated by the finding[8] that both processes responded in a similar manner to succinate and the adenine nucleotides, the response being determined by the type of mitochondrial preparation (fresh; intact or damaged by washing[12] at 0° C.; aged at 0° and 37° C.). Since for swelling to occur electron transport along the respiratory chain is required, and since intact mitochondria capable of phosphorylating do not swell, a labilizing change in the biochemical structure of isolated liver mitochondria suspended in deficient medium, leading to uncoupling, has been postulated as necessarily preceding mitochondrial swelling. Thyroxine appears to accelerate the latter change, which has been identified[9,12,13], albeit tenta-

tively, as caused by or to involve a loss of adenine nucleotides from the particles in the deficient medium lacking magnesium.

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Accelerated Recovery from Reserpine Depression by Monoamine Oxidase Inhibitors

IT is generally accepted that pretreatment with a strong monoamine oxidase inhibitor, for example iproniazid, antagonizes the behavioural depression induced by reserpine[1]. The converse experiment in which the inhibitor is given after reserpine in an attempt to terminate the depression apparently has not been as well explored; at least two papers, however, have reported failures to obtain such a reversal[2]. In the present experiments, chronically reserpinized animals were under observation for extended periods (weeks) before and after treatment with iproniazid or β-phenyl*iso*propylhydrazine (*JB* 516). The conditioning techniques used provided an objective assay of behaviour changes. Under these conditions, the behavioural depression produced by reserpine was clearly alleviated by a single injection of a monoamine oxidase inhibitor, although the onset of the arousal lagged about a day after the dosing.

Five hungry male rats, maintained at 80 per cent of their weight when feeding *ad libitum*, were trained to obtain a drop of sweetened condensed milk by pressing a lever in the wall of a small chamber. Not every press was rewarded; eligibility for reinforcement was programmed randomly and occurred, on the average, once a minute. This variable-interval reinforcement schedule produced a steady, high response-rate throughout the 1-hr. experimental sessions, and thus furnished a dependable behavioural base-line for the measurement of drug effects.

Administration of reserpine phosphate (0·2 mgm./kgm. base intraperitoneally) was begun after the lever-pressing rates had become stabilized. Injections were made immediately after every experimental session, even on days when other drugs were given. Behavioural tests were not run at week-ends, but the reserpine was injected every day once the series was started.

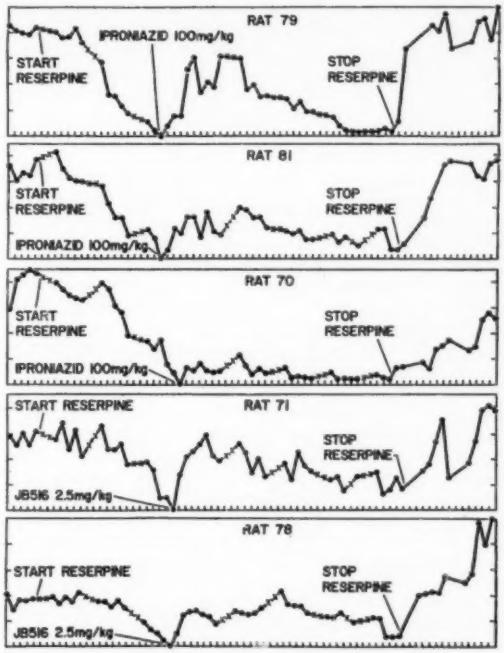

Fig. 1. Daily response output throughout the experiment for the five rats. Abscissa, daily 1-hr. test sessions: markers indicate successive days; ordinate, total number of lever responses: each marker denotes 400 responses. Reserpine was administered immediately after each test session on week-days and also on week-ends (indicated by crosses) when behavioural tests were not made. The first three reserpine injections were 0·5 mgm./kgm., and all succeeding ones, 0·2 mgm./kgm. A single dose of iproniazid or *JB* 516 was injected half an hour before the start of the test session indicated

After several reserpine injections with no apparent effect, the rates of response declined rapidly. When the rates were about 10 per cent or less of the pre-reserpine-levels, the monoamine oxidase inhibitors were administered. Three of the animals were injected with iproniazid phosphate (100 mgm./kgm. intraperitoneally) and two with β-phenyl*iso*propylhydrazine hydrochloride (2·5 mgm./kgm. intraperitoneally) half an hour before the start of the test period. Reserpine was injected as usual after this period, and daily for several weeks thereafter.

Fig. 1 gives the daily lever-pressing rates for the five animals throughout the course of the experiment. With the exception of rat 70 (which probably required a larger dose of iproniazid), substantial recovery from the reserpine inhibition may be observed on the second or third day after the dosing with the inhibitor; the reversal lasted about one month. Without the protection of an inhibitor, a reserpine series of this sort is usually lethal; the animals stop eating and seem to be less resistant to disease.

Also interesting was the observation that the monamine oxidase inhibitors actually increased the suppression of lever-pressing for many hours immediately after the dosing. In Fig. 1 it may be seen that on the day that the inhibitor was given, the response output of all animals approximated zero. Such absolute suppression of the lever-pressing rate within half an hour after an iproniazid or *JB* 516 injection has been observed invariably in more than twenty experiments. These doses of iproniazid and *JB* 516

have no obvious effects on the lever-pressing performance of rats not treated with reserpine.

These findings bear upon theories that attribute the behavioural effects of reserpine to the changes this drug brings about in the levels of endogenous brain monoamines; the serotonin and catechol amine in the brain are both depleted extensively and both have been taken to be implicated[1,2].

The present observation of a delayed reserpine reversal by the monoamine oxidase inhibitors follows the time-course of recovery of norepinephrine in the brain more closely than that of serotonin. Spector *et al.* have reported that the level of serotonin in the brain in reserpinized rabbits rose almost to normal within 2 hr. after an injection of *JB* 516, while the level of norepinephrine remained virtually unchanged[4]. During this time of recovered serotonin-levels in the present experiments, the inhibition of lever-pressing behaviour was not reversed, but actually was increased. The present observation that the peak of the recovery was delayed for a number of days corresponds with a similarly delayed increase in the norepinephrine-level in the brains of rabbits treated with monoamine oxidase inhibitors[4]. Also favouring an important role for the catechol amines are the findings that amphetamine, a close structural and pharmacological relative of the catechol amines, and cocaine, a known potentiator of catechol amine activity, both potently and rapidly reversed the behavioural inhibition produced by reserpine. Reversal of reserpine-induced depression of behaviour with cocaine has been observed by J. Seifter and one of us (L. S.) in experiments at the Wyeth Institute for Medical Research.

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Electrophoretic Mobilities of Normal and Regenerating Liver Cells

By measuring the electrophoretic mobility of cells, Ambrose, James and Lowick[1] have demonstrated that the average charge density of neoplastic kidney and liver cells is almost double that of the homologous normal cells.

It was further shown[2,3] that the increase in the negative charge density of tumour cells is due to a gradual alteration of surface properties in direct relation to the degree of malignancy.

These observations gave rise to the question as to whether the increased charge density of neoplastic cells is specifically characteristic of malignancy, or could perhaps be considered as one of the manifestations of normal growth.

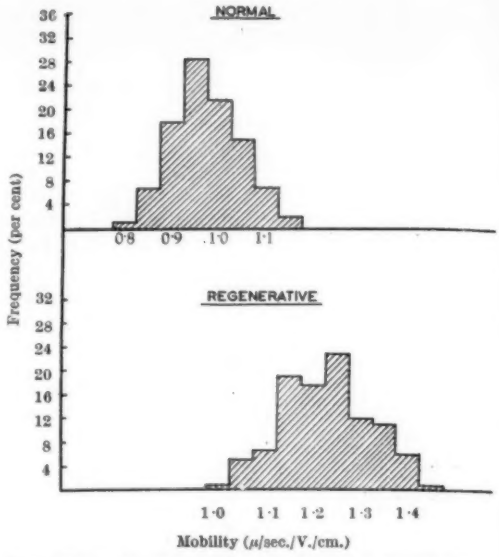

Fig. 1. Electrophoretic mobilities of single liver cells. Cells from normal liver show a mean mobility of 1·000 ± 0·015. Cell from regenerative liver 24 hr. after partial hepatectomy 1·257 ± 0·021 *S.E.*

In an attempt to shed light on this problem, the electrophoretic mobility of regenerating liver cells was compared with that of the homologous non-regenerating cells.

Cell suspensions of adult rat liver were prepared by perfusing the liver with 0·05 *M* ethylenediamine tetraacetic acid slightly homogenized in a loose-fitting Potter–Elvehjem glass homogenizer[4] and washed several times with phosphate-buffered saline[5]. This procedure yielded a rich suspension of liver cells. For electrophoretic measurements the cells were transferred to Sørensen's phosphate buffer *M*/15 (*p*H 7·4; ionic strength 0·174) and measured by the Northrop–Kunitz electrophoretic cell on a horizontal plane[6]. 100 cells, at least, were measured for each experiment. Erythrocytes served as internal control, and accordingly only animals in which the erythrocyte mobility was in the range of 1·21–1·27 μ/sec./V./cm. were used.

The measurements were carried out on coded samples so that the observer was unaware of the origin of the cells at the time of measurement.

Fig. 1 shows an increase by an average of 25 per cent in the mobility of the cells 24 hr. after partial hepatectomy, thus indicating the increased negative charge density of the regenerating liver cells.

When the changes in charge density of the cells during the regenerative process were followed up, an increase in mobility could already be observed at 6 hr. after the operation. The mobility was still high after 48 hr. However, in measurements taken after a considerable delay of 20 days, it was found that the cell mobility had returned to the normal level.

These experiments would indicate that the increased charge density of cells is also a manifestation of a normal growth process. It may be therefore that the growth associated with malignancy may be the cause of the increased charge density changes previously described[1,2] rather than a property specific to malignant cells.

A detailed account of this work will be published elsewhere.

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HÆMATOLOGY

Separation of Reticulocytes by means of Multi-Layer Centrifugation

Reticulocytes regarded as the youngest erythrocytes in the circulating blood have particular properties such as respiration[1,2], biosynthesis of hæm *in vitro*[3], electrophoretic mobility and osmotic resistance[4] different from those of mature erythrocytes. Separation of reticulocytes in a pure and physiological state seems necessary in studying their properties and functions[5].

We succeeded in separating reticulocytes by means of a multi-layer centrifugation with a gum acacia solution. The separating procedures are similar to those of leucocytes and bone marrow cells[6,7] reported previously. The technique depends on the difference in densities between reticulocytes and mature erythrocytes. Separating media with various degrees of specific gravity are prepared immediately before the experiment by diluting a stock solution with physiological saline solution. The stock solution is prepared as follows: about 30 ml. of distilled water is put into a flask, to which about 30–32 gm. of gum acacia powder is added and stirred with a glass rod. The stirring is continued while the flask is being put in a water-bath kept at 80–90° C. Then water is added slowly up to about 90 ml. After the powder is dissolved, it is centrifuged at 1,000*g* for 20 min. The supernate is diluted with distilled water to a density of 1·090 at 15° C. The density is measured by a hydrometer which covers the range 1·060–1·120 in steps of 0·001 unit. As this solution is hypotonic to blood cells, sodium chloride is added to keep the solution isotonic with blood cells—usually about 0·2 per cent. The *p*H of this solution is adjusted to 7·4 by adding a few drops of a saturated sodium hydroxide solution.

The stock solution can be stored for several weeks in a tightly closed container kept in a cold room. The proper dilution of the stock solution to secure desired densities for the separation of reticulocytes can be calculated from the following formula:

$$X = \frac{1 \cdot 090}{D - 1} \times A$$

where X is volume of physiological saline solution to be added, A is volume of stock solution, D is the density desired.

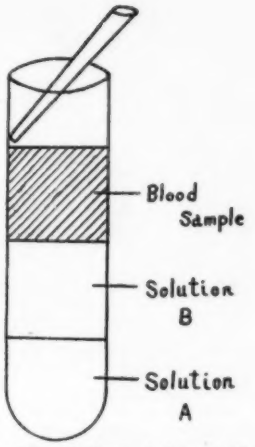

Fig. 1. Layered solutions before centrifugation

Usually two solutions A and B are used for separation; A with a specific gravity of 1·068–1·078 according to the preliminary test and B is 1·050 (15° C.). Each specific gravity desired should be determined by a preliminary test with blood cells, because there are individual and seasonal variations in density of reticulocyte.

Reticulocytosis is caused in adult rabbits weighing 2·5–3·0 kgm. by repeated bleeding or administration of phenylhydrazine; thus 10 ml. of blood per kgm. body-weight is withdrawn daily from the ear vein or by heart puncture until the reticulocyte count reaches about 30 per cent; or 0·3 ml. of a 2·5 per cent phenylhydrazine hydrochloride solution per kgm. body-weight is injected subcutaneously daily until the reticulocyte count reaches about 50 per cent. The blood of the rabbit with reticulocytosis is withdrawn from the ear vein in measured amounts of 3·8 per cent sodium citrate or neparin solution. The blood is washed with physiological saline solution and then centrifuged at 1,000*g* for 20 min. The supernate and buffy coat (a concentrated leucocyte and platelet layer) are pipetted off.

The sedimented red blood cells are suspended in a two- or three-fold volume of physiological saline

Fig. 2. Separated blood cells in a centrifuge tube. 1, Fibrin layer with leucocytes and platelets; 2, reticulocyte layer; 3, mature erythrocyte layer

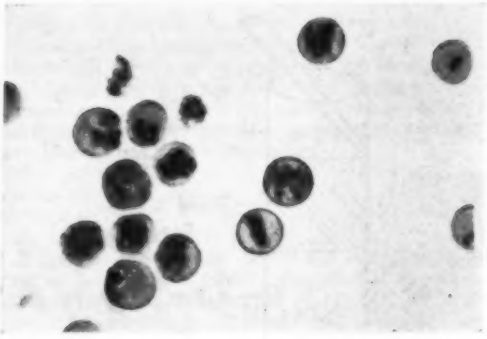

Fig. 3. Separated reticulocytes (brilliant cresyl blue staining). (× 800)

solution and used for a multi-layer centrifugation. All the procedures are carried out in a cold room kept at 2°–0° C.

Two ml. of solution *A* is poured into a centrifuge tube (13 mm. in inner diameter, 10 ml. in capacity) and 2 ml. of solution *B* is layered over the *A* carefully along the inner wall of the tube with a pipette. Then about 3 ml. of the blood sample is put on *B* (Fig. 1). The layered solutions are centrifuged at 4,000–5,500*g* for 15 min. A Marusan electric centrifuge (horizontal head) is employed: conical head centrifuges do not seem convenient for a multi-layer centrifugation. After centrifugation the following three layers are formed in a centrifuge tube (Fig. 2): from the top to the bottom, (1) fibrin lying with a few leucocytes and platelets; (2) reticulocyte (immature erythrocyte) layer; (3) mature erythrocyte layer. The reticulocytes float on the boundary of solutions *A* and *B* forming a stratum, so that they can be easily collected with a pipette. The reticulocytes thus prepared show a 95–100 per cent purity in separation (Fig. 3). Their functions and properties will be discussed in the *Japanese Journal of Physiology*.

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Human Embryo Hæmoglobin

Although evidence of the presence of a second fœtal hæmoglobin in young human fœtuses is accumulating[1–6], uncertainty still seems to prevail in some quarters as to the existence of this embryonic hæmoglobin[7]. The following report of a paper-electrophoretic study of the hæmoglobins of six 6–12-week-old and five 15–18-week-old human fœtuses may therefore be of some interest.

Hæmolysates containing about 10 per cent hæmoglobin were prepared from citrated cord blood follow-

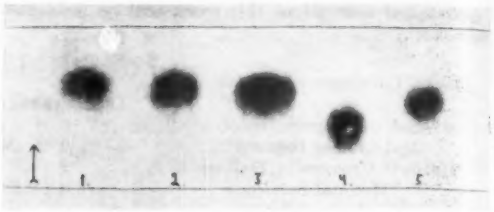

Fig. 1. Electrophoretic pattern of hæmoglobins. 1, 2 and 3, normal adult hæmoglobin; 4, hæmoglobin from 12-week-old fœtus; 5, normal full-term new-born infant. Arrow indicates direction of migration

Fig. 2. Electrophoretic pattern of hæmoglobins. 1, Mixture of hæmoglobin from 12-week-old fœtus and full-term new-born infant; 2 and 4, hæmoglobin from 12-week-old fœtuses; 3, normal full-term new-born infant. Arrow indicates direction of migration

ing the technique of Singer *et al.* The spots were run on Whatman No. 1 filter paper for 20–21 hr. in barbital buffer at a *p*H of 8·6 and at approximately 0·1 ionic strength.

As can be seen from Table 1 and Figs. 1 and 2, the migration of the hæmoglobins in the group of 7–12-week-old fœtuses was definitely slower than the migration of the hæmoglobins in the group of 15–18-week-old fœtuses and of full-term infants and adult controls. In relation to the migration of normal adult hæmoglobin the mean ratio fœtal hæmoglobin : adult hæmoglobin was 0·86 in the group of small fœtuses and 0·98 in the group of 15–18-week-old fœtuses.

After migration a mixture of hæmoglobins from a 12-week-old fœtus and a full-term new-born infant showed signs of separation (Fig. 2).

It seems reasonable to assume that the hæmoglobin in the blood of the 7–12-week-old fœtuses described here is identical with the embryonic

Table 1. Migration (cm.) of Hæmoglobins from Six 7–12-week-old and Five 15–18-week-old Human Fœtuses. Whatman No. 1. Veronal Buffer *p*H 8·6, 20–21 hr., 4·5–6 m.amp.

Age of fœtus, weeks	Migration (cm.) of: Fœtal hæmoglobin	Adult hæmoglobin	Fœtal hæmoglobin/adult hæmoglobin	Mean
7 (6 cm.)	7·6	8	0·94	
8 (6 cm.)	7·4	8	0·92	
10 (8 cm.)	7·5	8·7	0·86	0·86
11 (10 cm.)	6·0	7·0	0·85	
12 (9 cm.)	4·5	5·0	0·9	
12 (9 cm.)	3·5	5·0	0·7	
15 (16 cm.)	8·5	8·7	0·97	
16 (16 cm.)	8·3	8·1	1·02	
18 (20 cm.)	8·0	8·5	0·94	0·98
18 (22 cm.)	7·8	8·0	0·98	
18 (21 cm.)	8·0	8·2	0·98	

hæmoglobin of a 10-week-old human fœtus which Halbrecht and Klibansky identified by means of paper electrophoresis. The "primitive type *P*" embryonic hæmoglobin described by Allison[2] and the early embryonic hæmoglobin distinguished through alkali denaturation by Drescher and Künzer[1] may well be identical with the hæmoglobin of the small fœtuses reported here.

The migration pattern of the hæmoglobins of the 15–18-week-old fœtuses differed significantly from the hæmoglobins of the 7–12-week-old group. The mobility of the hæmoglobins of the older group of fœtuses showed but small differences as compared with the hæmoglobin of normal full-term infants.

From the results of this investigation it was thus possible to distinguish a human embryonic hæmoglobin from a group of 7–12-week-old fœtuses, which, on paper electrophoresis under the conditions described, showed a mobility definitely slower than that of the hæmoglobin of normal full-term infants. The mobility of the hæmoglobins of the group of 15–18-week-old fœtuses did not differ significantly from that of normal full-term infants.

These findings support the view[4] that the hæmoglobin in the erythrocytes derived from the mesoblastic tissue during early embryonic life is of a different type from the hæmoglobin of hepatic and medullary origin. If this holds true, most of the hæmoglobin of mesoblastic type is replaced by hæmoglobin of hepatic origin in the course of 15–18 weeks of fœtal life.

I am indebted to the Sigrid Jusélius Foundation for a grant towards the cost of this investigation.

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PATHOLOGY

Leucocytic Emigration from Inflamed Capillaries

THE precise manner in which margination and emigration of leucocytes occur in acute inflammation has not been elucidated despite numerous careful studies with the light microscope. Utilizing electron microscopy we have observed the emigration of leucocytes from capillaries in areas of acute inflammation in dog pancreas. Capillary endothelial cells react to injury by forming intra-lumenal finger-like projections of cytoplasm. These projections insinuate themselves around, and envelop, adjacent leucocytes, and, in this manner, draw the latter into the capillary wall (Fig. 1). This intramural localization of leucocytes is equivalent to 'margination' as seen by light microscopy and would appear to occur by a process similar to that of phagocytosis. The leucocytes are then extruded through the antiluminal border of the endothelial cell where they are separated from the extravascular space only by the basement membrane

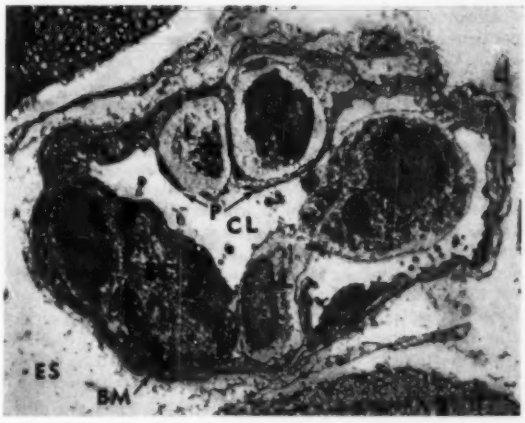

Fig. 1. Electron photomicrograph of an inflamed capillary in which leucocytes (*L*) are surrounded by endothelial cytoplasmic processes (*P*). Lumen of capillary (*CL*), endothelial cell (*EC*), basement membrane (*BM*), extravascular space (*ES*). (× *c.* 4,670)

of the endothelial cell. After separation of leucocyte and endothelial cell by as much as 300 Å., new basement membrane forms between them and is continuous with the old. Simultaneously old basement membrane adjacent to the leucocyte becomes smudged and frayed and gradually melts away, releasing the leucocyte into the extravascular space.

Only rarely are interruptions seen in the continuity of either endothelial cell or basement membrane during the passage of leucocytes through them. In most instances, both endothelial cell and basement membrane are continuous and are re-formed behind the leucocyte before either is breached ahead of it. That the process is highly selective is demonstrated by the fact that segmented leucocytes are involved predominantly. The nature of this selectivity was not determined in the present work; it may be related to humoral factors acting on either the leucocyte or the endothelium. Agents which affect the degree or extent of inflammatory exudation may act on the endothelial cell.

The exact processes of margination and emigration described here may not occur in all species and for all inflammatory stimuli. Preliminary observations suggest that species variations may exist. Under the conditions of the experiment described here, however, the endothelial cell appears to play a very active part in these processes. Full details of this work will be published in the *American Journal of Pathology*.

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Blood Corticotrophin in Cushing's Syndrome

PREVIOUS work on the level of blood corticotrophin in cases of Cushing's syndrome has been scanty and has not involved a full-scale bioassay of concentrated extracts used at sufficiently high dose-levels. Some of the early work on this subject was reviewed by Loraine[1] and semiquantitative values for four Cushing's patients have been given by Clayton[2].

From our experiments, values are given for the corticotrophin content of blood from two patients with Cushing's syndrome and from normal and hypertensive subjects.

Corticotrophin was extracted from large volumes of blood (500–1,000 ml.), collected between 10 and 11 a.m. The blood was run directly into glacial acetic acid to prevent possible enzymic destruction of the hormone[2]. In order to concentrate it for bioassay the corticotrophin was extracted by adsorption on oxycellulose followed by elution with 0·1 *N* hydrochloric acid by Astwood's method[4] as modified for blood by Sydnor and Sayers[5]. The latter obtained an 85 per cent recovery of corticotrophin added to hypophysectomized rat blood. In our experiments, the eluate was stirred with moist 'De-acidite *FF*' resin (carbonate form), sufficient being added to bring the *p*H to 2·7. The solution was then freeze-dried, stored dry over phosphorus pentoxide at 4° C. and was re-dissolved in 0·9 per cent saline, acidified with a trace of 0·01 *N* hydrochloric acid, for intravenous injection into rats.

The blood concentrates were assayed by their action in depleting adrenal ascorbic acid in hydrocortisone-treated rats[6–8]. Our assays involved the use of 30 rats and compared the effect of three dose-levels of the blood concentrates with three dose-levels (0·13, 0·40, 1·2 mU.) [1 milliunit (1 mU.) = 1/1,000 I.U.] of the 2nd International Standard for corticotrophin. The highest dose of the concentrate was equivalent to at least 50 ml. of blood. The accuracy was satisfactory for a bioassay method, average limits of error calculated at the 5 per cent level being 58–165 per cent. Results are given in Table 1.

Table 1. Corticotrophin Content of Human Blood

Type of patient		Sex and age (yr.)	Volume of blood concentrated (ml.)	Potency of blood concentrate in rat assay	Limits of error expressed as percentage of the potency†	Blood corticotrophin mU.‡/100 ml. blood
Normal*	I	1 *M* 30 1 *F* 34 1 *F* 37	1,080	0·77	43–131	0·71
	II	1 *M* 35 1 *M* 40	1,000	0·58	43–232	0·71
Hypertensive		*M* 51	1,000	0·59	55–183	0·72
Cushing's case 1		*F* 38	750	1·31	74–135	2·16
Cushing's case 2		*M* 44	450	0·81	74–136	1·80

* Blood was pooled from 3 and 2 individuals.
† Calculated at 5 per cent level.
‡ 1 mU. = 1/1,000 I.U.

In normal subjects, Sydnor *et al.*[9] and Paris *et al.*[10] believed from their experimental work that the corticotrophin content of blood was less than 0·5 mU./100 ml. Levels of 30–200 mU./100 ml. reported by Bornstein *et al.*[11] have been criticized by Loraine[1], who suggested that these levels would have been accompanied by clinical features of adrenocortical hyperfunction.

It seems unlikely that the high values for our two Cushing's patients are caused by hypertension (see Table 1). Clearly more cases should be studied in greater detail, with values before and after treatment correlated with the pathology of the adrenal and pituitary glands.

This research programme has been supported by a grant to one of us (A. R. C.) from the Scottish Hospital Endowments Research Trust.

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RADIOBIOLOGY

A Sexual Difference in the Concentration of Iodine-131 by the Submaxillary Gland of Mice

A sexual dimorphism in the submaxillary gland of mice has been described by Lacassagne[1]. In the male the secretory tubuli are more developed than in the female. This difference has since been reported as depending on endocrine glands[2–4].

Human saliva concentrates iodine-131 (ref. 5). Since this demonstration, experiments have been carried out on the iodine-concentrating capacity of salivary glands. Logothetopoulos and Myant[6] established this fact for the submaxillary gland of mice and it was shown that the degree of concentration is related to the strain of the animals[7]. Radioautographic studies[8] led to the conclusion that the secretory tubuli are the structures of the gland which concentrate iodine.

Table 1

Total iodine-131 per mgm. of submaxillary tissue / Total iodine-131 per mgm. of blood				
Males		Females		*P*
No. of animals	Mean ± *S.E.*	No. of animals	Mean ± *S.E.*	
61	5·25 ± 0·31	40	3·89 ± 0·32	< 0·01

In the present communication we report our findings on the sexual difference in the iodine-concentrating capacity of submaxillary glands of mice.

Sixty-one male and forty female *C3H* mice were injected intraperitoneally with 5 μc. of carrier-free solution of iodine-131. 1 hr. later the submaxillary gland was removed and blood collected under ether anæsthesia. The glands and blood were weighed and radioactivity measured in a well counter. The ratio:

$$\frac{\text{total } ^{131}\text{I per mgm. submaxillary tissue}}{\text{total } ^{131}\text{I per mgm. blood}}$$

was taken as a measure of the degree to which submaxillary tissue is able to concentrate iodine.

The results are given in Table 1.

These results show that there is a sexual difference in the capacity to concentrate iodine by the submaxillary gland of mice.

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Uptake of Magnesium-28 by the Skeleton of a Sheep

In order to find the cause of hypomagnesæmia in adult ruminants, it is necessary to have a better understanding of the mechanism controlling magnesium-levels in blood and particularly the relationship between bone and blood magnesium. Bone from cows suffering from hypomagnesæmia showed no depletion of magnesium[1], and this has led to the theory that the lability of bone magnesium is much reduced in adult animals[2]. There are no figures in the literature, however, for either the amount or the rate of mobilization of skeletal magnesium in adult ruminants, but such data can be obtained from work on the uptake of radioactive magnesium by bone. Because of the short half-life of magnesium-28, only that part of the bone magnesium which is rapidly exchanged with the magnesium of plasma and other body fluids can be measured.

After six days on a constant diet of grass nuts containing 1·2 gm. of magnesium, a five-year-old Greyface wether, weighing about 65 kgm., was given 40–60 μc. carrier-free magnesium-28 as the chloride into the jugular vein. 10 hr. later, immediately after sampling the blood, the sheep was killed by exsanguination under nembutal anæsthesia, and duplicate samples of bone, free so far as possible from blood and bone marrow, were taken from various parts of the skeleton and their radioactivity determined the same day in a well-type scintillation counter.

The values obtained for the calcium–magnesium ratio and specific activity of the samples of selected bones are given in Table 1. The specific activities are expressed both as the percentage of dose × 10^3 per mgm. of total magnesium and as the percentage of the specific activity of the plasma, the latter being a measure of the proportion of bone magnesium which has exchanged with the magnesium of body fluids during the 10-hr. period.

A marked variation in specific activity from bone to bone was observed and was greater in regions of rapid bone metabolism than in compact bone. Thus

Table 1. Ratio of Calcium to Magnesium in the Selected Bones and their Uptake of Magnesium-28 at 10 hr. after Intravenous Administration

Bone	Calcium/magnesium	Specific activity (percentage of dose) (× 10^3 per mgm. magnesium)	Specific activity (as percentages of specific activity of plasma)
Femur shaft	57	0·048	0·86
Femur epiphysis	52	0·130	2·3
Rib shaft	56	0·079	1·4
Rib sternal end	46	0·129	2·3
Lumbar vertebra	46	0·099	1·7

the sternal end of the rib and the epiphysis of the femur showed the greatest exchange and the femur shaft the lowest.

The over-all percentage of bone magnesium in equilibrium with the magnesium in the body fluids was low; the values for the samples of individual bones ranged from 0·86 to 2·3 per cent. The limiting factor may be the exchange-rate itself or the amount of bone magnesium which is available for exchange. It is interesting to note that the variation in specific activity of individual bones was in the same order as the degree of magnesium depletion in the corresponding bones from magnesium-depleted calves[8].

There are no analytical figures in the literature for the magnesium content of the skeleton of either adult cattle or sheep. Estimates can be made from the calcium content of the skeleton of dairy cows[4] and sheep, and the calcium–magnesium ratio in bone. The calculated values for cows range from 90 to 160 gm. depending on the size of the animal, and for sheep from 8 to 10 gm.; the figures for sheep are based on only three analyses. If the reserves of bone magnesium are assumed to be the same as the percentage of bone magnesium which has later reached equilibrium with the magnesium of body fluids, namely, 2 per cent, the skeleton of dairy cows contains 1·8–3 gm. of magnesium which can be released to satisfy physiological needs, that is, enough for 3–5 gallons of milk. In the case of sheep, the reserves (160–200 mgm.) are equivalent to the endogenous fæcal loss for 1 day[5]. The lactating animal may be more efficient in mobilizing bone magnesium than the non-lactating animal, since the resorption of bone mineral to provide the calcium necessary for lactation will also release magnesium[6]. This effect, however, will be small because of the wide ratio of calcium to magnesium in bone and the fact that the release of calcium from bone is small, for example, less than 20 gm. a day for dairy cows[6].

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BIOLOGY

Effect of Chelating Agents on Plasmodium Formation by the True Slime Mould, *Didymium nigripes*

Spores of *Didymium nigripes*, a true slime mould, germinate to liberate myxamœbæ which multiply by binary fission. After a period of exponential growth, the uninucleate myxamœbæ fuse to form macroscopic, multinucleate veins of rhythmically flowing protoplasm called plasmodia. Following an indefinite period of growth, the plasmodia differentiate into spore-containing fruiting bodies. Kerr and Sussman[1] grew *D. nigripes* in monoxenic culture with *Aerobacter aerogenes* on a dilute glucose–peptone–yeast extract agar medium buffered with phosphate at *p*H 6·2 (hereafter called GPY/5). All stages of the life-cycle were regularly reproduced in less than 7 days. When 0·25 per cent (w/v) brucine was added to the GPY/5, plasmodia did not appear. Instead, the myxamœbæ remained indefinitely as such and could be sub-cultured as amœbæ. The myxamœbæ quickly formed plasmodia when harvested from the GPY/5-brucine plates and washed by differential centrifugation.

The morphogenesis of uninucleate myxamœbæ to multinucleate plasmodia is one of the most interesting, but least understood, stages in the life-cycle of the true slime moulds. According to Cadman[2] and Ross[3], plasmodium formation involves the pair-wise fusion of myxamœbæ as gametes to form zygotes. Presumably the zygotes develop into plasmodia by growth, nuclear division, and fusion with other zygotes. Since an understanding of this morphogenesis is an essential prerequisite to studies on the genetic life-cycle, and to studies of heterokaryosis and nuclear-cytoplasmic interrelationships in the plasmodium, an elucidation of its mechanics has been undertaken. The mechanism by which brucine inhibits plasmodium formation was studied in the hope that it would yield information on the normal process of plasmodium formation.

One possible action of brucine might be to stimulate the bacterial associate to produce a metabolite which in turn would inhibit plasmodium formation. This possibility was tested by growing myxamœbæ on GPY/5-brucine with a variety of bacterial associates, including *Escherichia coli*, *Sarcina lutea*, *Pseudomonas fluorescens* and *Aerobacter aerogenes*. Plasmodia did not appear in any instance. It therefore seemed probable that brucine was acting directly on the myxamœbæ rather than via the bacterial associate.

Several lines of evidence indicated that brucine might be inhibiting plasmodium formation by chelating with ions in the medium. First, although the medium used was saturated by 0·1 per cent (w/v) brucine, the inhibitory action of brucine became more and more complete as its concentration was raised to 0·25 per cent. This might be expected if brucine were acting as a chelating agent, but not if it were acting as a metabolic inhibitor. Second, brucine inhibits the formation of plasmodia rather than plasmodia themselves, for if plasmodia are sub-cultured on GPY/5-brucine, they grow and fruit normally. Third, the density of myxamœbæ profoundly influences the number of plasmodia which they form. Even in the absence of brucine, plasmodium formation was delayed when the myxamœbæ quickly reached a high density. Plasmodia appeared in greatest number when the myxamœbæ exhausted their bacterial food supply while still at a relatively low density. Brucine-inhibition was complete only when the myxamœbæ covered the entire plate at high density before exhausting their food supply; when myxamœbæ were plated clonally on GPY/5-brucine, occasional plasmodia appeared.

As a result of these observations, experiments were devised to test the hypothesis that multivalent cations are essential to plasmodium formation. The results are reported in Table 1. Chelating agents were added to GPY/5 media which were spread with spores of *D. nigripes* and 0·3 ml. of a broth culture of *A. aerogenes*. In each case the *p*H was adjusted to 6·0–6·5. The plates were incubated at 22° C. for 4 days and then scored for growth of myxamœbæ and appearance of plasmodia. Tetrasodium ethylenediamine tetraacetic acid at 10^{-3} *M* completely prevented the formation of plasmodia, even when the spores gave rise to clones. Either 10^{-3} *M* Mn^{++} or 10^{-3} *M* Fe^{++} reversed the inhibition of ethylenediamine tetraacetic acid to permit numbers of plasmodia to form. None of the other ions tested was effective. Other chelating agents, including sodium citrate, were also effective in inhibiting plasmodium formation.

Table 1. The Growth of Myxamœbæ and Appearance of Plasmodia on GPY/5 Media to which Various Agents had been added

Addition	Amœbæ	Plasmodia
Control	+++	+++
0·25 per cent (w/v) brucine	+++	0
0·1 *M* Sodium citrate	++	0
10^{-3} *M* Ethylenediamine tetraacetate acid	++	0
5×10^{-4} *M* Ethylenediamine tetraacetic acid	+++	+
10^{-3} *M* Ethylenediamine tetraacetic acid		
+ 10^{-3} *M* calcium chloride	++	0
+ 10^{-3} *M* strontium chloride	++	0
+ 10^{-3} *M* magnesium chloride	++	0
+ 10^{-3} *M* manganese chloride	++	++
+ 10^{-3} *M* ferrous chloride	++	++

The experimental results thus confirm the hypothesis that multivalent cations are necessary for plasmodium formation. A likely mode of action of brucine is as a chelating agent, removing these ions from the medium.

The development of a technique which will measure plasmodium formation in a quantitative manner is in progress. These experiments will be reported elsewhere.

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Effect of Extracts from Fucoids in promoting Settlement of Epiphytic Polyzoa

In a previous communication[1] it was suggested that the simple ciliated larvæ of some species of sedentary organisms might respond to qualitative differences in bacterial films formed on surfaces immersed in the sea, as was suggested by Wilson to explain the behaviour of the larvæ of *Ophelia bicornis* Savigny[2].

Table 1. Settlement of *Alcyonidium polyoum* on Treated Inert Surfaces

Type of surface	No. settled in experiment (1)	(2)	(3)	Total No. of settled larvæ
Unfilmed surface	5	3	3	11
Surface filmed by standing in sea water for 24 hr.	1	4*	0	5
Surface similarly filmed and treated with extract of *Fucus serratus* in distilled water	90	46	26†	162

* Treated with distilled water for 2 hr. after filming.
† Extract of *Fucus* made up in sea water.

Table 2. Ratio of Number of Larvæ of *Alcyonidium polyoum* settled on Films treated with Algal Extracts (n_A) in Comparison with Settlement on Films treated with *F. serratus* (n_F)

Algal extract of:	Ratio n_A/n_F	
Fucus serratus	1·00	Probably effective
Fucus vesiculosus	1·04	
Ascophyllum nodosum	1·72	
Pelvetia canaliculata	0·63	
Fucus spiralis	0·09	Not effective
Laminaria digitata	0·25	
Ulva lactuca	0·04	
Gigartina stellata	0·00	
Control	0·22	

Some recent experiments with the larvæ of the polyzoan *Alcyonidium polyoum* (Hassall) have confirmed this. This species normally occurs on the fronds of *Fucus serratus* L., and its larvæ settle preferentially on fronds of this and related fucoids when offered a choice of different algal fronds[3]. When these larvæ were placed in a glass vessel and offered small rectangular pieces of inert material for settlement, as described in a previous communication[1], they tended to avoid filmed surfaces, as did the larvæ of *Bugula flabellata*, though they did not settle very readily on unfilmed surfaces. However, when the filmed surfaces were treated beforehand with an extract of *Fucus serratus* they become very attractive to the larvæ, as can be seen from Table 1. Extracts of other algæ were tested in the same way, but only certain species were regularly effective in promoting settlement. It can be seen from Table 2 that those species which promoted settlement all belonged to the Fucaceæ. Of the most attractive sources, *Fucus serratus* L., *Fucus vesiculosus* L. and *Ascophyllum nodosum* (L.) le Jol, only *F. serratus* normally bears colonies of *Alcyonidium*. Perhaps the other two fucoids occur too high up the shore, or do not liberate the active principle on to the surface of the thallus. Similar results have also been obtained using the larvæ of the closely related polyzoan *Flustrellidra hispida* (Fabricius).

The settlement of these larvæ seems not to be due to any physical property of the film, such as its thickness, since, in the absence of *Fucus* extract, filmed surfaces are not attractive. However, when unfilmed surfaces were briefly treated with the extracts they did not attract the larvæ strongly; only filmed panels could be made artificially favourable for settlement.

The bacterial film is therefore necessary to promote good settlement, but it must be treated with the extract. The simplest hypothesis is that the active principle is transferred to the film, and, as it diffuses out, it attracts the larvæ. Other mechanisms which may lead to larval recognition are possible, however; for example, the existing bacterial film may modify substances present in the *Fucus* extract, or the bacterial flora of the film itself may be altered in the presence of an extract of *Fucus*. The mechanism by which the larvæ of *Alcyonidium polyoum* and *Flustrellidra hispida* recognize the treated film is now being investigated, while similar phenomena are being sought in the settlement behaviour of other marine larvæ.

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Sex Hormones as a Possible Factor influencing the Level of Parasitization in Frogs

Large numbers of frogs (*Rana temporaria*) have been examined for helminth parasites during the period 1956–60. On analysing the results of these investigations, it was found that the level of parasitization in male frogs during and immediately before the breeding season was considerably higher than that in females. This difference between the levels of parasitization in the two sexes was particularly marked in the case of the trematodes, *Polystoma integerrimum* and *Gorgoderina vitelliloba*, the nematode, *Rhabdias bufonis*, and the acanthocephalan, *Acanthocephalus ranae*. After the breeding season, the difference became less marked. This is evident from Table 1, which is based on the results obtained by examining approximately 500 frogs from a single locality, over a period of four years.

Table 1. Levels of Parasitization of Male and Female Frogs during and outside the Breeding Season

Species of parasite	Percentage of frogs parasitized during January and February		Percentage of frogs parasitized during April and May	
	Males	Females	Males	Females
Trematodes				
Polystoma integerrimum	14·0	8·1	16·1	17·0
Gorgoderina vitelliloba	10·2	5·3	12·8	7·6
Dolichosaccus rastellus	8·9	10·2	5·3	6·2
Haplometra cylindracea	49·6	41·8	53·0	44·4
Nematodes				
Rhabdias bufonis	52·3	29·4	47·8	37·4
Oswaldocruzia filiformis	71·6	82·3	76·9	74·2
Cosmocerca ornata	86·3	94·1	88·4	92·3
Acanthocephala				
Acanthocephalus ranae	13·4	8·0	22·5	24·0

Similar, although less conclusive, results have been obtained by Markov[1] and Mazurmovich[2].

The difference in parasitization-levels of male and female frogs, well marked in the breeding season and much less well marked at a time of the year when the gonads have suffered regression, may be explained by assuming that the female sex hormones depress the level of parasitization in some way or other. In order to test this particular hypothesis we took fifty male frogs from a population known to be heavily parasitized with helminths, and injected half of them with œstradiol benzoate (B.P.) in 20 per cent ethyl oleate and 80 per cent arachis oil. The other half were regarded as controls. The experimental series were given 0·5 mgm. of œstradiol benzoate in 0·1 mgm. doses over a period of 12 days. At the end of three weeks both the experimental and control frogs were killed and examined for helminths.

Table 2. Comparison of Parasitization-levels in Male Frogs treated with Œstradiol and Untreated Frogs

Species of parasite	Percentage of control frogs parasitized	Percentage of experimental frogs parasitized
Trematodes		
Polystoma integerrimum	16	4
Gorgoderina vitelliloba	24	12
Dolichosaccus rastellus	12	0
Haplometra cylindracea	56	40
Nematodes		
Rhabdias bufonis	64	32
Oswaldocruzia filiformis	80	72
Cosmocerca ornata	90	72
Acanthocephala		
Acanthocephalus ranæ	20	8

The results of this examination are given in Table 2. The results of these preliminary experiments suggest that the presence of the female hormone, œstradiol, in the tissues and fluids of the host does lead to a depression of the level of parasitization by helminths. We propose to investigate this phenomenon in more detail.

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Mobility of Barnacles

If a young barnacle is removed from a surface the remains of the antennal suckers, marking the point at which the individual cemented itself as a cypris larva, may sometimes be found near the centre of its base. It has generally been assumed that this point of basal attachment is fixed. The position selected by the barnacle, when a larva, would then always be included within the outline of its base, though not necessarily at the centre, for if growth were not radially symmetrical the original point of attachment might become eccentric.

Recent experiments have been carried out in which the growth of operculate barnacles has been followed after they have settled on transparent plates. These observations show quite clearly that such animals possess a limited mobility when subjected to lateral pressure. If one side of a young colony is cleared of settlement and kept bare, the individuals forming the free edge of the colony will be pushed outwards by the growth of the rest. This pressure, exerted over a period of several months, can displace the outermost individuals a few centimetres from their original position. Fig. 1 shows how a young individual can be moved through several times its own diameter during the first six months of life. Evidently the cement layer between the hypodermis and the solid surface must slowly be sheared as it allows the shell and membranous base to glide along. The advancing edge of the base can form new adhesions to the substratum in the same way that it does during the expansion of the base in normal growth. Rotation of the barnacle in response to currents probably occurs also in the same manner[1]. Mobility has been observed both on smooth and on uneven surfaces.

Balanus balanoides, in common with other barnacles, has very strong powers of adhesion, so that when a number of individuals of about equal size

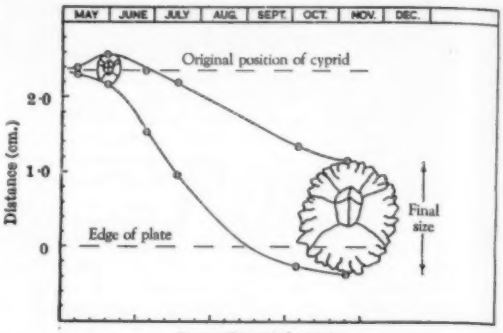

Fig. 1. Illustration of the movement of an individual *Balanus balanoides* (L.) which had settled at a distance of 2·4 cm. from the edge of a flat plate and was subjected to lateral pressure from other individuals growing above it (not shown). The ordinate gives the distance from the edge of the plate of the carinal and rostral ends of the base of the barnacle, the distance separating the lines representing its basal diameter. The abscissa gives the time which elapsed after settlement. By September the individual illustrated had begun to be forced over the edge of the plate

come in contact their bases remain attached to the solid surface and their sides become pressed together. Continued growth is then possible only upwards, unless the peripheral individuals can be slid outwards, when limited expansion of the whole colony may occur under its own two-dimensional pressure.

When the individuals which come into contact are not of the same age or do not belong to the same species, the result may be quite different. One specimen, usually the older, remains firmly attached, while the other is gradually displaced upwards on to the advancing shell plates of the former. This process can be readily observed when later settlements of *Elminius modestus* insinuate themselves among fast-growing spat of *Balanus balanoides*. The former are so completely squeezed out that they may come to project above the general level and can be detached by a gentle blow. If such individuals survive, their elevated position has some advantages, allowing more rapid growth and earlier maturity[2], and tending in the end to cause the underlying individuals to become silted up and smothered.

The ability to adhere strongly to a surface, but at the same time to retain sufficient mobility to move a short distance or to readjust themselves to alterations in the shape of the substratum, has two obvious advantages. It allows barnacles to grow on such surfaces as the shells of other animals, where there are slow relative movements or a gradual expansion of the whole area, without the danger of losing their adhesion. It also allows them, provided that the original settlement was sufficiently heavy, to expand into nearby bare spaces on a solid surface which were not, for some reason, initially colonized.

Most barnacles, within their normal geographical range, produce a superabundance of planktonic larvæ, which settle in greater numbers than is necessary for survival. Consequently the factor which chiefly limits the size of populations is shortage of settling space. Each species must therefore, so far as possible, occupy all the surface suitable to it and exclude competing organisms. It is in this respect that the faculty of limited mobility is of such importance. Without it a colony of barnacles would be able to occupy only a small area around each of the positions initially selected by the cyprids, and

competing organisms would be quite free to settle afterwards in any of the spaces left unoccupied. Without such mobility young individuals growing among larger ones would be easily lifted off or crushed as the older ones crowded them in; this indeed may sometimes happen[3]. The limited degree of sliding and adjustment possessed by barnacles therefore confers an advantage both to established populations, which are able to expand over any available space, and also to invading populations of new spat, which may in consequence be able to form a second tier of individuals using their competitors as an alternative, though mobile, substratum. The outcome of such a struggle between species depends not only on settlement pattern, population density and relative growth-rates, but also on the forces operating between the growing edge of the shell of each species and the substratum with which they are both in contact.

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Mechanism of Change in Permeability in Living Membranes

AN important feature of many excitable cells is that their permeability to ions and even to large molecules such as aldolase depends on the potential difference across the cellular membrane[1]. Thus, in nerve fibres a depolarization leads to a transitory increase in sodium permeability (P_{Na}), often followed by a slower development of an increase in potassium permeability (P_K). More recent studies of skeletal[2,3] and cardiac (Carmeliet, E., and Weidmann, S., personal communication) muscle have demonstrated an opposite relation between P_K and membrane potential that has been designated 'anomalous rectification'. The purpose of this communication is to point out the possibility that the mechanism of these changes in permeability may be related to that recently proposed on the basis of monomolecular film studies of drugs known to affect the ionic permeability of natural membranes[4–6]. This mechanism is based on recently demonstrated physico-chemical principles and is susceptible to experimental test.

Agents such as local anæsthetics, designated as 'stabilizers', decrease the permeability of living membranes or depress their ability to undergo the rapid increase in permeability during excitation[1,6]. They interact with monolayers of lipoidal molecules (for example, stearic acid) and thereby add their own bulk to the films; thus, molecular packing is increased, as reflected by a rise in surface pressure[5–9]. Conversely, compounds called 'labilizers' or 'unstabilizers', such as certain veratrum alkaloids, increase membrane permeability[1,6]. These gradually remove the lipoidal molecules from the monomolecular films and hence reduce packing and surface pressure[4,5]. It has been proposed from such observations and others that the degree of packing of membrane molecules surrounding a site of ion passage (for example, an intermolecular space) is related inversely to (*a*) the ease of ion transport, and especially (*b*) to the ability of ion transfer to increase during excitation[1,5,6]. The concept of changes in surface pressure through effects in the region adjacent to the locus of ion penetration focuses attention on membrane organization, which must be mesomorphic, that is, intermediate to a liquid and a solid[10]. Previous research has suggested this type of structure[6]; our recent work also suggests this, for the antagonistic effects of stabilizers and labilizers became apparent in the monolayers when the films are compressed to a condition intermediate to that of a liquid and solid[5].

The work on drugs has led to emphasis on electrostatic and van der Waals interactions between the membrane and extrinsic molecules as the basis for changes in packing and in permeability. The possibility of such changes with membrane potential arises if the natural membranes contain lipophilic (hydrophobic) cations and anions at low concentration. Guastalla *et al.*[11] have already shown that relatively weak electrical gradients (V./cm.) can govern the accumulation of such ions at an oil–water interface, the speed of the process depending on the intensity of the applied field. Natural membranes, with a thickness of the order of 100 Å., have across them a 'resting potential' of about 100 mV. and hence an electric field, E, of 100,000 V./cm.; the inner boundary is normally negative to the exterior[6].

Because of their nature, the lipophilic ions would not leave the membrane readily; therefore they would be distributed by the field as shown diagrammatically in Fig. 1*A*. The sketch on the left indicates only the ions capable of interacting with membrane molecules in the regions of sodium transfer, that on the right only those able to affect potassium transport. A_1 represents a small anion, perhaps with several charges, C a large cation with fewer charges, and A_2 an anion comparable in bulk and in number of charges to C. One more assumption is inherent in the model, namely, that only the outermost interface, M, possesses the mesomorphic state required for the permeability changes. The deeper region of the membrane is regarded as essentially fluid, thereby serving as a reservoir of lipophilic ions without contributing directly to the permeability changes. Experimental evidence for a superficial layer as the basis of permeability change in living membranes has recently been presented by Shanes and Berman[12], who also direct attention to other indications for this in the literature. The concentrations of the ions on the inner boundary of the interface regulate their degree of interaction with M.

Fig. 1*B* indicates an intermediate stage in the redistribution of the hydrophobic ions during a sudden depolarization of the membrane. A_1, by virtue of size and charge, responds most rapidly to the alteration in E. As it moves out of M, P_{Na} increases. If the depolarization is sustained, as shown in the lowermost part of Fig. 1, C becomes more uniformly distributed and so enters M and reduces P_{Na} again. Such a secondary decrease in P_{Na} is well known and has been designated 'inactivation'. Finally, as A_2 leaves M, P_K increases. If, in addition to or instead of A_2, cations were available comparable to C but more effective and able to enter the regions of potassium transport, then instead of the more familiar increase in P_K (and membrane conductance) with depolarization, that is, delayed rectification, a decrease would be obtained, that is, anomalous

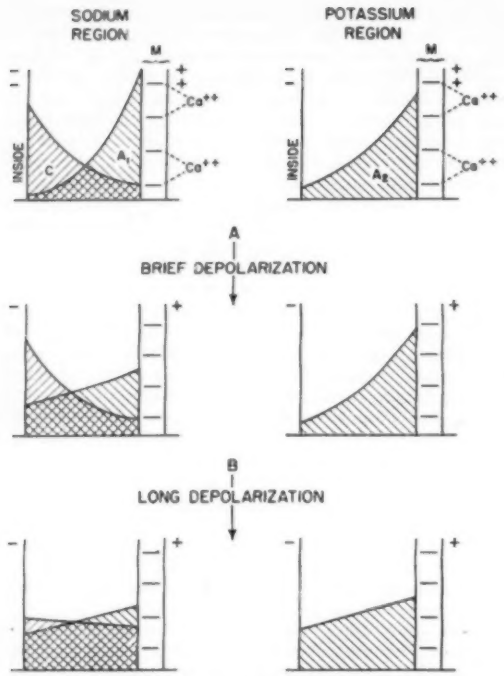

Fig. 1. Hypothetical representation of the distribution of lipophilic cations and anions in a resting, normally polarized excitable membrane (*A*) and during two successive periods of a moderate depolarization. *M* is an outer mesomorphic region susceptible to change in permeability by interaction with drugs or with the lipophilic anions (A_1, removal of which increases P_{Na}, and A_2, removal of which increases P_K) and cations (*C*, entry of which causes inactivation, that is, a secondary decrease in P_{Na}). The negative sites in *M* are molecular groups that contribute to cation selectivity; they are shown as partly occupied by calcium in the normally polarized cell

rectification. From this point of view, then, anomalous rectification is similar to the inactivation process for sodium, but apparently more effective inasmuch as permeability to potassium is lowered below the resting level. However, this difference in the inactivation processes might be merely a reflexion of a deficit in A_2, a deficit which makes P_K at rest comparable to P_{Na} after A_1 has been partly removed. S. Weidmann's observation (personal communication) that potassium depolarization produces relatively little increase in the membrane conductance of Purkinje fibres appears in keeping with this.

It would be premature to contend that all changes in permeability can be ascribed to one principle. For example, it is possible that at least part of the increase in permeabilities during depolarization is a consequence of removal of calcium ions from superficial sites concerned with ion passage[13]. Such removal could increase permeability by allowing the monovalent ions to penetrate at these points; in addition, it is possible that calcium removal exposes adjacent negative groups of neighbouring molecules in the membrane, which are thereby driven apart to admit sodium and potassium ions and perhaps large molecules as well. In keeping with these proposals, lowering of *p*H also accomplishes a reduction in ion permeability[4]. Note, however, another possible role for calcium (and hydrogen) ions in Fig. 1. By neutralizing negative groups of the membrane molecules in *M*, calcium could reduce the repulsion of anions and so enhance the entry of A_1 and A_2 into *M*, thereby lowering the permeability to sodium and potassium; concurrently, the attraction for cations would be reduced, thereby decreasing the entry of *C* into *M* and hence suppressing inactivation. This provides an electrostatic basis for the similarity between calcium deprivation and depolarization in respect of their effects on permeabilities and on inactivation[14,15].

It is necessary to set up working models employing the principles that have been outlined to determine to what extent the kinetics of changes in permeability in living systems can be duplicated. A possible test of the validity of the proposed hypothesis would be to determine whether the effects of various lipophilic ions, for example, tetraethylammonium and tetrabutylammonium, which have been striking in some excitable cells[16,17], give evidence of being modified in a predictable manner by alterations in membrane potential.

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Colchi-Autotetraploidy in *Adiantum capillus-veneris*

Ferns are well known for possessing polyploid complexes, but we do not know of any instance where Howard and Manton's[1] method has been used for analysis of their genome relations and elucidation of their nature of polyploidy. The primary reason for this appears to be the difficulties involved in raising autotetraploids in ferns. So far the standard practice has been to raise such strains by apospory[2]. Manton[3] alone made a meiotic analysis of the autopolyploid series in *Osmunda regalis* L. following this method and found reasonably high degree of multivalents.

The aposporous method of raising autopolyploids has certain inherent limitations in that it is laborious, time-consuming and above all it is uncertain. On the other hand, colchicine treatment is the most sure method available at present for inducing polyploidy. However, so far there has been a solitary attempt by Panigrahi[4], who applied colchicine to the young embryos of *Cyclosorus repandulus* (v.A.v.R.) Ching

at the three-leaved stage, but could only obtain a sectorial chimera as evidenced by root tip smears while no fronds were borne on it. We have treated with colchicine the rhizomes of a few locally available ferns. Besides easy manipulation, the rhizome treatment gives quick results and eliminates the long time that is usually required for the sporophytes to reach maturity in case of spore, sporeling and embryo treatments. Of the three methods tried (immersion in aqueous solution of colchicine, lanolin paste and injections) we find the immersion in 0·5 per cent solution for nearly 48 hr. to be uniformly successful. The rhizomes were not defoliated because otherwise the first leaves produced would be sterile and also the rhizomes usually die after treatment when defoliated. The present account deals with the results on *Adiantum capillus-veneris* L. Here the new fronds start appearing within about three weeks after treatment. A morphological comparison with the controls treated similarly with distilled water revealed that autotetraploids, though smaller in size, show the usual *gigas* characters (thickness of rachis and lamina, stomatal size).

During meiosis the controls show invariably 30 bivalents (Fig. 1). The autotetraploids possess quadrivalents and bivalents. An analysis of ten exceptionally clear cells reveals that the number of quadrivalents ranges from 13 to 10 and the bivalents from 34 to 40. The average associations are $12_{IV} + 36_{II}$ (Fig. 2). The quadrivalents are in the form of rings or chains and possess almost completely terminalized chiasmata at diakinesis. The high degree of terminalization may also augment the normal disjunction during anaphases. In one case two bivalents showed early disjunction. Meiosis is perfectly normal and apparently normal spores result. In *Osmunda regalis*[3] the percentage of quadrivalents per cell is nearly 66, while in the present case it is about 40, and trivalents are absent. The difference may be due to the smaller size of the chromosomes in the latter.

As indicated above, the spores are well filled and apparently normal. Sixty-four spores result in each sporangium. Interesting enough is the presence among them of about 3–5 per cent spores with bilateral symmetry, while the rule for the species and the genus is tetrahedral. Perhaps this may be due to the colchicine action. It is pertinent to mention here that Mehra and Loyal[5] have observed another aberration, namely, branched rhizoids, being caused by colchicine action in the sporelings of *Goniopteris prolifera* Roxb. The change in symmetry of the spores is not due to the polyploid condition, because no such aberration has been found in any of the polyploid ferns discovered so far in Nature.

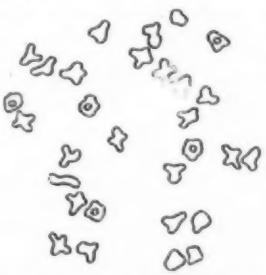

Fig. 1. Diakinesis in spore-mother-cell of diploid *Adiantum capillus-veneris*, $n = 30$. (× 1,000)

Fig. 2. Diakinesis in spore-mother-cell of autotetraploid *Adiantum capillus-veneris*, showing $13_{IV} + 34_{II}$. (× 600)

The present findings reveal that the rhizome treatment has obvious advantages over the uncertain method of apospory. Here in the meristem there is a single apical cell, the products of which give rise to the leaf and root primordia. Hence once the apical cell is rendered polyploid, all the subsequent fronds will naturally be autopolyploids, some of which will be fertile. The resulting spores should normally yield diploid gametophytes which can be used for crossing with natural tetraploids.

The occurrence of perfectly normal disjunction and well-filled and apparently normal spores by the autotetraploid in *Adiantum capillus-veneris* is rather important. The reason for this may lie in the fact that the present species is diploid throughout its range, and that in the genus there already exist potentialities for polyploidy up to the hexaploid[4] (apogamous) level. Furthermore, the heterozygous nature of this horticultural fern is an additional point in favour. These are the two important points that are usually taken into consideration before embarking on a programme of induced autopolyploidy.

Apart from assessing the role of autopolyploidy in the evolution of ferns, the autopolyploids will also be useful in producing autotriploids from which trisomic lines for genetic studies may be raised. Their use in biosystematic studies of the fern polyploid complexes is of course unquestioned. The details of this work will appear elsewhere.

We are indebted to Prof. P. N. Mehra for advice, and to Dr. T. N. Khoshoo for criticism.

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MICROBIOLOGY

Alleged Transformation of Yeast

CRUDE preparations of deoxyribonucleic acid from one yeast strain, called the donor, sometimes appear to confer fermentation properties of the cell on a second strain, the acceptor, when added to the growth medium[1-3]. For example, when a yeast which did not ferment sucrose was grown in wort containing a preparation of deoxyribonucleic acid derived from a sucrose-fermenting strain, the transfer of part of the culture to a bijou bottle containing sucrose dissolved in yeast water (3 per cent 'Difco' yeast extract in water) resulted in fermentation. Single-cell isolates obtained from the treated yeast, which had been serially sub-cultured through the same medium, also fermented sucrose, and on crossing with the original acceptor strain gave Mendelian segregation of sucrose-fermenting power. These observations were explained[1-3] by assuming that nuclear transformations akin to that already observed in various bacterial species[4-9] had been induced in the acceptor yeast by the deoxyribonucleic acid from the donor. In view of the potential importance of such transformation from both the technological and scientific points of view an attempt has been made to investigate this phenomenon further.

The yeast strains used were those originally employed by Oppenoorth[3]. The acceptor strains included (1) *R7*, a yeast which was originally isolated by Winge and Roberts as a descendant of crossings between strains of *Saccharomyces cerevisiae*, *Saccharomyces chevalieri* and *Saccharomyces italicus*, and (2) *S. chevalieri K83S58*. Both acceptor yeasts fermented galactose and glucose but neither fermented raffinose or sucrose. The donor strain was *S. chevalieri W332*, which fermented both raffinose and sucrose.

Preparations of deoxyribonucleic acid were obtained from 48-hr. cultures of the donor yeast grown in shake culture at 25° C. in either wort or a medium consisting of yeast extract (Difco, 0·3 per cent), malt extract (0·3 per cent), peptone (0·5 per cent) and glucose (1·0 per cent) in water. The extraction procedure for deoxyribonucleic acid and the method of dissolving the product in wort were as described by Oppenoorth[3]. As will emerge, a critical finding was that the sterilization procedure used for the solution of deoxyribonucleic acid affected the result. The solution was sterilized either by (*a*) filtration through a glass filter (porosity *S*) or (*b*) filtration through a Seitz filter containing a Ford 'Sterimat' pad (grade *SB*). A solution (3·0 ml.) of ethanolic hydrogen chloride, prepared by diluting concentrated hydrochloric acid (8·0 ml.) to 100 ml. with ethanol, was added to aliquot portions (1·0 ml.) of the solution of deoxyribonucleic acid before and after sterilization. After standing for 15 min. the resulting precipitate was removed by centrifugation, washed with ethanolic hydrogen chloride and dissolved in water for estimation of deoxyribonucleic acid content by the method of Seibert[10]. It was afterwards found that sterilization by method (*a*) had no significant effect on the concentration of deoxyribonucleic acid, but sterilization by method (*b*) completely removed the deoxyribonucleic acid from solution in wort.

The effects of the solutions obtained by methods (*a*) and (*b*) on the acceptor strains were then investigated. In a typical case a series of McCartney bottles containing wort (4·0 ml.) or the test solution (4·0 ml.) was inoculated from a 1-day culture of yeast *R7* grown in wort. The yeast was incubated with shaking at 25° C. for 4 days. After growth the wort was removed by centrifugation and the residual yeast suspended in water (0·2 ml.). Aliquot portions (0·04 ml.) of the suspensions were then transferred in duplicate to bijou bottles, individually containing sucrose (2 per cent) dissolved in yeast water, while the remaining yeast was washed four times with water. The latter yeast was suspended finally in water (0·12 ml.) and duplicate portions (0·04 ml.) were transferred to sucrose medium as before. At the same time a portion of the washed yeast suspension was serially diluted with water to give cell concentrations of approximately 1×10^8, 5×10^5 and 1×10^3 cells/ml. and portions (0·02 ml.) of these suspensions were inoculated on to a solid medium prepared by the incorporation of agar (Davis 'Nutrient', 2 per cent) into the synthetic medium of Harris and Thompson[11] and using sucrose (0·5 per cent) as the source of carbon instead of glucose. The donor yeast grew satisfactorily on this medium while the acceptor yeasts failed to grow although they were viable as shown by growth when glucose was the carbon source. The bottles and plates were incubated at 25° C. and examined at intervals over a period of 30 days. The ability of the yeast to grow on the synthetic medium containing sucrose as sole carbon source was also investigated by means of the haemocytometer slide-culture technique of Gilliland[12], which more accurately reflects the ability of cells to grow than the conventional plating techniques. In this instance, a portion of the washed yeast was suspended in the synthetic medium containing gelatin (6 per cent) and incubated on the slide at 18° C. for 6 hr. The slides were then examined microscopically for micro-colonies arising from sucrose-fermenting strains. The results (Table 1) show that: (1) the ability of the acceptor yeast to ferment sucrose was lost by thorough washing; (2) the presence of deoxyribonucleic acid in the growth medium was not necessary for a positive fermentation result to be observed; and (3) the ability of the yeast to ferment sucrose was not reflected in its ability to grow on solid medium with sucrose as sole carbon source.

Table 1. SUCROSE FERMENTATION BY ACCEPTOR YEASTS TREATED IN DIFFERENT WAYS

Growth medium	Treatment of yeast	Fermentation result*	
		Yeast *R7*	Yeast *K* 85858
Wort	washed	none	none
Wort	unwashed	none	none
Wort/deoxyribonucleic acid Seitz filtered	washed	none	none
Wort/deoxyribonucleic acid Seitz filtered	unwashed	+ (after 2 days)	+ (after 2 days)
Wort/deoxyribonucleic acid glass filtered	washed	none	none
Wort/deoxyribonucleic acid glass filtered	unwashed	+ (after 2 days)	+ (after 2 days)

* No growth was observed on sucrose plates or sucrose–gelatin slides

A possible explanation of the results was that invertase was present in the deoxyribonucleic acid preparations used. To investigate this possibility, portions (0·01 ml.) of the deoxyribonucleic acid/wort solution were added to bijou bottles containing sucrose or raffinose (2·0 per cent) dissolved in yeast water. After incubating for 24 hr. at 25° C. the sugars present were examined by chromatography using a mixture of ethyl acetate, pyridine and water (10 : 4 : 3, v/v) as developing solvent and the dip

reagent of Harris and MacWilliam[12] for detecting the sugars. It was thus found that considerable hydrolysis of sucrose and raffinose to monosaccharides had in fact occurred. Clearly, the deoxyribonucleic acid preparation was contaminated with invertase in sufficient quantity to yield monosaccharides responsible for the positive fermentation tests observed in the earlier work. Moreover, the enzyme passed through the Seitz filters used and gave rise to the positive fermentation tests even in the absence of deoxyribonucleic acid. It is therefore apparent that such fermentation tests are insufficient in themselves to indicate transformation. The further evidence for transformation presented earlier, namely, the isolation of single cells capable of fermenting sucrose or raffinose from subcultures originally not possessing this faculty, is not conclusive. In this connexion, the presence of hydrolytic enzymes in the test medium will allow growth to occur on the monosaccharides produced when the yeast is sub-cultured through a series of bijou bottles containing the test polysaccharide. In these circumstances cells capable of fermenting the polysaccharide might arise by mutation and subsequent selection even though controls not containing the deoxyribonucleic acid–enzyme mixture as used by Oppenoorth[2] fail to produce mutants of this type. It appears therefore that the evidence presented for transformation of yeasts in the manner suggested is capable of alternative explanation, and that further investigation is required to substantiate the earlier claims.

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VIROLOGY

Plaque Formation by an Orphan Virus of Fowls

When Sharpless *et al.*[1] and Fontes *et al.*[2] reported the adaptation of fowl lymphomatosis virus (*RPL*.12) to chicken embryo tissue culture, and later when plaque assay methods were described for this virus[3–5], there was promise of an *in vitro* system for the study of viral oncogenesis. However, the suspicion that this virus may not in fact be the causal agent of fowl lymphomatosis was aroused when it was found that the inoculation of high-titre tissue culture material into chickens of a stock known to be susceptible to the *ES*4 strain of fowl lymphomatosis virus was not followed by the formation of tumours even after six months. Moreover, when these birds were challenged with *ES*4 virus, they were found to be completely susceptible (Carr, J. G., Macpherson, I. A., and Stoker, M. G. P., unpublished results). It was also shown that a high dilution of pooled normal sera from the *ES*4 susceptible flock neutralized plaque formation by the virus in tissue culture. A possible explanation of these results is that the virus we were studying was a fowl orphan virus present in poultry stocks. Since then confirmation has been received in personal communications from B. R. Burmester, G. R. Sharpless and R. J. Huebner, respectively, giving results of extensive investigations in the United States. These show that the virus is enzootic in many flocks.

Fig. 1

Despite its apparent lack of relationship to fowl lymphomatosis this ubiquitous and hardy virus may be of considerable interest in its own right. The purpose of this communication is to describe an improved method of plaque assay which is more sensitive and gives considerably better resolution than the techniques previously reported. It has the additional advantage that it can be adapted for rough assays of both virus and neutralizing antibody.

The method was based on that described by Cooper[6]. Base layers of 4 ml. containing 10 per cent calf serum, 10 per cent tryptose phosphate broth (Difco) and 0·9 per cent agar (Difco) in Eagle's medium were prepared in 60-mm. Petri dishes. When the base layers had set they were overlayed with 2·5 ml. of the same medium containing 0·1 ml. of virus dilution and 3×10^7 freshly trypsinized liver cells from 17-day-old chick embryos. On the fourth day of incubation 2·5 ml. of 0·01 per cent neutral red in agar medium was added and plaques (Fig. 1) counted daily until a maximum count was obtained 3–4 days later.

Table 1. Relative Sensitivities of Plaque Techniques for a Fowl Orphan Virus

Method	Relative sensitivity
Agar suspension technique as described in the text with :	
(1) Chick embryo liver	100
(2) Chick embryo kidney	100
(3) Whole chick embryo	4
(4) Young adult fowl liver	65
Monolayers of chick embryo liver (ref. 4)	13

The relative sensitivity of this method compared with other systems is given in Table 1. Although chick embryo kidney cells were found to be as sensitive as liver cells, the latter were preferred since higher yields could be obtained. For rough assays 0·1 ml. of virus dilution or virus/antibody mixture was added to 0·9 ml. of agar medium held at 44° C. and 0·02 ml. of this agar dilution was allowed to set on a base layer. A 60-mm. Petri dish accommodates 6–8 spots. When set, the spots were overlayed with liver cells in agar and then treated as described above. Since discrete plaques can be distinguished in these spots a direct measure of infectivity is obtained.

It will be seen from Fig. 1 that there was a continuous gradation of plaque size (0·5 mm.–5 mm. diameter). When cloned virus from large and small plaques was plated, the same wide distribution of plaque size was obtained from each clone. Incubation of virus and cells in fluid suspension for 2 hr. before plating did not lead to greater uniformity of plaque size. Buthala also found several sizes of plaques when this virus was plated on monolayers of chicken embryo kidney. The explanation of this effect is not known.

B. R. Burmester, G. R. Sharpless and A. K. Fontes[7] have found that this virus is in some ways similar to the adenoviruses of man and have suggested that it be called *GAL* (gallus, adenolike).

I wish to express my thanks to Prof. M. G. P. Stoker for his helpful advice and criticism.

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Effect of Ultrasonic Vibration on the Hæmagglutinating Activity of Some Para-influenza Viruses

The effect of sound vibration on influenza virus has been described previously[1,2]. Lief and Henle[2] showed that sonic treatment of influenza virus neither increased the hæmagglutinin titre nor changed the ratio of the hæmagglutinin titres obtained with chicken and guinea pig erythrocytes. The results briefly reported here deal with the effect of ultrasonic vibration on the hæmagglutinating activity of some para-influenza viruses.

Different para-influenza viruses were cultivated in cultures of calf or monkey kidney cells. Culture fluids containing large amounts of infectious virus were centrifuged at 2,500 r.p.m. for 30 min. The supernatants were subjected to ultrasonic vibration at about 20 kc./sec. for 10 min. in an *MSE* Mullard ultrasonic disintegrator. The hæmagglutinin tests were generally performed at 4° C. employing guinea pig and chicken erythrocytes.

Table 1. Guinea Pig Hæmagglutinin Titres of Untreated and Ultrasonic-treated Viruses

	Incubation	Para-influenza-1 Sendai	Para-influenza-2 *CA*	Para-influenza-3 *Umed**	*SA* *S 1*†
Untreated	4° C.	8	2	16	16
Treated	4° C.	128	16	128	256
Untreated	37° C.	128	8	128	64
Treated	37° C.	256	16	256	256

* A Swedish bovine strain.
† A Swedish strain of the myxovirus *SA*, described by Schultz and Habel (ref. 4).

The results presented in Table 1 show that if the hæmagglutinin tests were performed at 4° C., the guinea pig hæmagglutinin titres increased 8–16 times after ultrasonic treatment of virus. If, however, the hæmagglutinin tests were performed at 37° C., the guinea pig hæmagglutinin titres were higher and did not increase to the same extent after treatment.

The rise in guinea pig hæmagglutinin after ultrasonic treatment was not accompanied by an increase of chicken hæmagglutinin (Table 2). Similar observations have been made with influenza virus treated with ether[3]. To ascertain whether the effect of ultrasonic vibration was due to a release of non-infectious and less-sedimentable hæmagglutinin, untreated and treated virus samples were centrifuged at 25,000 r.p.m. for 1 hr. in a Spinco preparative centrifuge. The supernatants and sediments were titrated for hæmagglutinin and infectivity. The results of this experiment are recorded in Table 3, which shows that the level of hæmagglutinin was 16 times greater in the supernatant of the treated virus than in that of the untreated virus. Furthermore, the ratio guinea pig hæmagglutinin/TCD_{50} was about 100 times greater in the supernatant than in the sediment of ultrasonic-treated virus.

Table 2. Hæmagglutinating Activity of Untreated and Ultrasonic-treated Virus

Virus	Untreated		Treated	
	GPHA	CHA	GPHA	CHA
Sendai	8	128	128	128
CA	2	2	16	2
Umed	16	<2	128	<2
S 1	16	128	256	186

GPHA, Guinea pig hæmagglutinin; CHA, chicken hæmagglutinin.

Table 3. Guinea Pig Hæmagglutinin Titres of Para-influenza Virus centrifuged at 25,000 r.p.m. for 1 hr.

Titre/ml.	Untreated		Ultrasonic treated	
	Supernatant	Sediment	Supernatant	Sediment
GPHA	8 (8)*	32 (256)	128 (128)	256 (256)
log TCD_{50}	5·9	7·9	5·7	8·1

* After ultrasonic treatment.

The effect of ultrasonic vibration on the hæmagglutinating activity of para-influenza viruses may be due to a disaggregation of a non-infectious hæmagglutinin. Further investigations dealing with this hypothesis will be published later.

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FORTHCOMING EVENTS

Monday, January 2

PLASTICS INSTITUTE (in the Lecture Theatre of the Institution of Electrical Engineers, Savoy Place, London, W.C.2), at 2.30 p.m.—Mr. H. A. Thomas: "Plastics and Textiles" (Young People's Lecture).

SOCIETY OF CHEMICAL INDUSTRY, LONDON SECTION (14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Mr. H. F. Corsan: "Financial Management in the Chemical Industry".

Wednesday, January 4

BRITISH OCCUPATIONAL HYGIENE SOCIETY (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 10 a.m.—Meeting on "Health Physics".

SOCIETY OF CHEMICAL INDUSTRY, CORROSION GROUP—CATHODIC PROTECTION PANEL (at 14 Belgrave Square, London, S.W.1), at 6 p.m.—Discussion of "Galvanic Anodes". Opening Speakers: Dr. G. E. Gardam, Mr. P. V. Palmer and Mr. K. P. Scott.

BRITISH INSTITUTION OF RADIO ENGINEERS, RADAR GROUP (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 6.30 p.m.—Mr. W. E. Brunt: "Automatic Techniques in Civil Air Line Communications Systems".

Thursday, January 5

INSTITUTION OF MECHANICAL ENGINEERS, INDUSTRIAL ADMINISTRATION AND ENGINEERING PRODUCTION GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 10.30 a.m.—Informal Discussion on "The Reliability of Mechanical Engineering Parts of Data Processing Systems".

ROYAL AERONAUTICAL SOCIETY (at 4 Hamilton Place, London, W.1), at 3 p.m.—Mr. R. J. Lees: "Gyroscope and Pendulum—the Modern Travel Agents" (Young People's Lecture).

ROYAL INSTITUTION OF NAVAL ARCHITECTS (at 10 Upper Belgrave Street, London, S.W.1), at 4.45 p.m.—Mr. A. Hunter, O.B.E.: Third Amos Ayre Lecture.

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, W.C.2), at 5.30 p.m.—Dr. J. S. Forrest: "The Application of Electronics to the Electricity Supply Industry" (Third Hunter Memorial Lecture).

INSTITUTE OF METALS, LONDON LOCAL SECTION (at 17 Belgrave Square, London, S.W.1), at 6.30 p.m.—Dr. J. E. Hughes: "Metallurgy in Electronics".

ROYAL PHOTOGRAPHIC SOCIETY OF GREAT BRITAIN, MEDICAL GROUP (at 16 Princes Gate, London, S.W.7), at 7 p.m.—Mr. Charles Engel and Mr. John Brace: "Closed Circuit Television in Medical Diagnosis, Research and Teaching".

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

ASSISTANT LECTURER in PSYCHOLOGY IN THE DEPARTMENT OF PHILOSOPHY—The Registrar, University College, Singleton Park, Swansea (January 14).

LECTURER IN PHYSICS (EXPERIMENTAL); a LECTURER IN THEORETICAL PHYSICS; and ASSISTANT LECTURERS IN PHYSICS—The Registrar, The University, Manchester 13 (January 14).

RESEARCH ASSISTANT (with some knowledge of metallurgical techniques), for work in the metal physics group under the direction of Dr. P. B. Hirsch—The Secretary to the Department of Physics, Cavendish Laboratory, The University, Free School Lane, Cambridge (January 15).

LECTURER IN VETERINARY HISTOLOGY AND EMBRYOLOGY—The Secretary of University Court, The University, Glasgow (January 18).

CHAIR OF MECHANICS—The Registry, The Old Schools, The University, Cambridge (January 21).

SCIENTIFIC OFFICER or SENIOR SCIENTIFIC OFFICER (with a good honours degree, preferably with postgraduate experience), to work on biochemical processes in seeds and seedlings—The Secretary, National Vegetable Research Station, Wellesbourne, Warwick (January 21).

LECTURER or ASSISTANT LECTURER IN STATISTICS—The Registrar, The University, Leeds 2 (January 24).

SENIOR LECTURER or LECTURER IN MATHEMATICS IN THE FACULTY OF ENGINEERING, University College of the West Indies, Trinidad—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (January 25).

LECTURER IN THE DEPARTMENT OF FOOD TECHNOLOGY at the University of New South Wales—The Agent-General for New South Wales, 56 Strand, London, W.C.2, and The Appointments Section, The University of New South Wales, Box 1, Post Office, Kensington, New South Wales, Australia, marking envelope "University Appointment" (Australia and London, January 27).

LECTURERS or ASSISTANT LECTURERS IN SOCIAL ANTHROPOLOGY, GEOLOGY, MATHEMATICS and PHYSICS at the University College of Rhodesia and Nyasaland—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (January 30).

SENIOR LECTURER (preferably with research interest in the application of mathematics to some engineering subject) IN ENGINEERING MATHEMATICS—The Secretary, The Queen's University, Belfast, Northern Ireland (January 30).

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PLANT BREEDERS (2) (with a degree in agriculture or science or an equivalent) within the University of Sydney, to carry out wheat breeding at the North-West Wheat Research Institute, Narrabri—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, January 31).

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UNIVERSITY LECTURER (with special qualifications in applied thermodynamics, and preferably an interest in heat transfer apparatus and some industrial or research experience) IN MECHANICAL ENGINEERING—The Secretary, The University, Aberdeen (January 31).

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AGRONOMIST or PLANT PHYSIOLOGIST (honours graduate in science or agricultural science with relevant postgraduate research experience, and preferably prior experience with tobacco) at the Tobacco Research Institute, C.S.I.R.O., Mareeba, North Queensland, to investigate factors affecting the nitrogen status of the tobacco plant, together with various problems in tobacco agronomy arising in North Queensland—Chief Scientific Liaison Officer, Australian Scientific Liaison Office, Africa House, Kingsway, London, W.C.2, quoting Appointment No. 815/29 (February 11).

CHAIR OF GEOLOGY tenable in the Newcastle Division of the University of Durham—The Registrar, University Office, 46 North Bailey, Durham (February 11).

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CHAIR OF APPLIED MECHANICS tenable in the Newcastle Division of the University of Durham—The Registrar, University Office, 46 North Bailey, Durham (February 18).

ASSISTANT EXPERIMENTAL OFFICER (with a degree in science or diploma in horticulture or agriculture), to assist in field experimental work—The Secretary, National Vegetable Research Station, Wellesbourne, Warwick.

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RESEARCH ASSISTANT (preferably with an honours degree in physical and inorganic chemistry) IN THE DEPARTMENT OF MINERALOGY AND PETROLOGY, for a programme of research concerning fundamental solid-state problems in the field of cement chemistry—Dr. J. D. C. McConnell, Department of Mineralogy and Petrology, Downing Place, Cambridge.

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SENIOR TECHNICIAN or TECHNICIAN (with instrument or physics workshop experience) IN THE RADIOTHERAPY DEPARTMENT, for duties which include Physics Workshop, Isotope Laboratory, Radium and Mould Room work—The Group Secretary, Romford H.M.C., Oldchurch Hospital, Oldchurch Road, Romford, Essex, quoting Ref. 17/39.

REPORTS and other PUBLICATIONS

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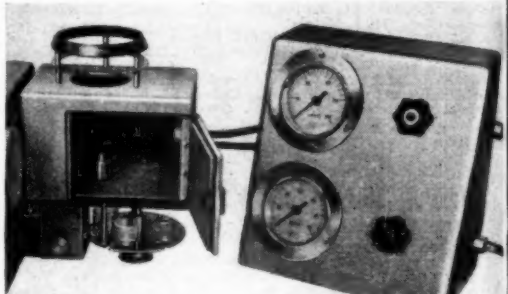

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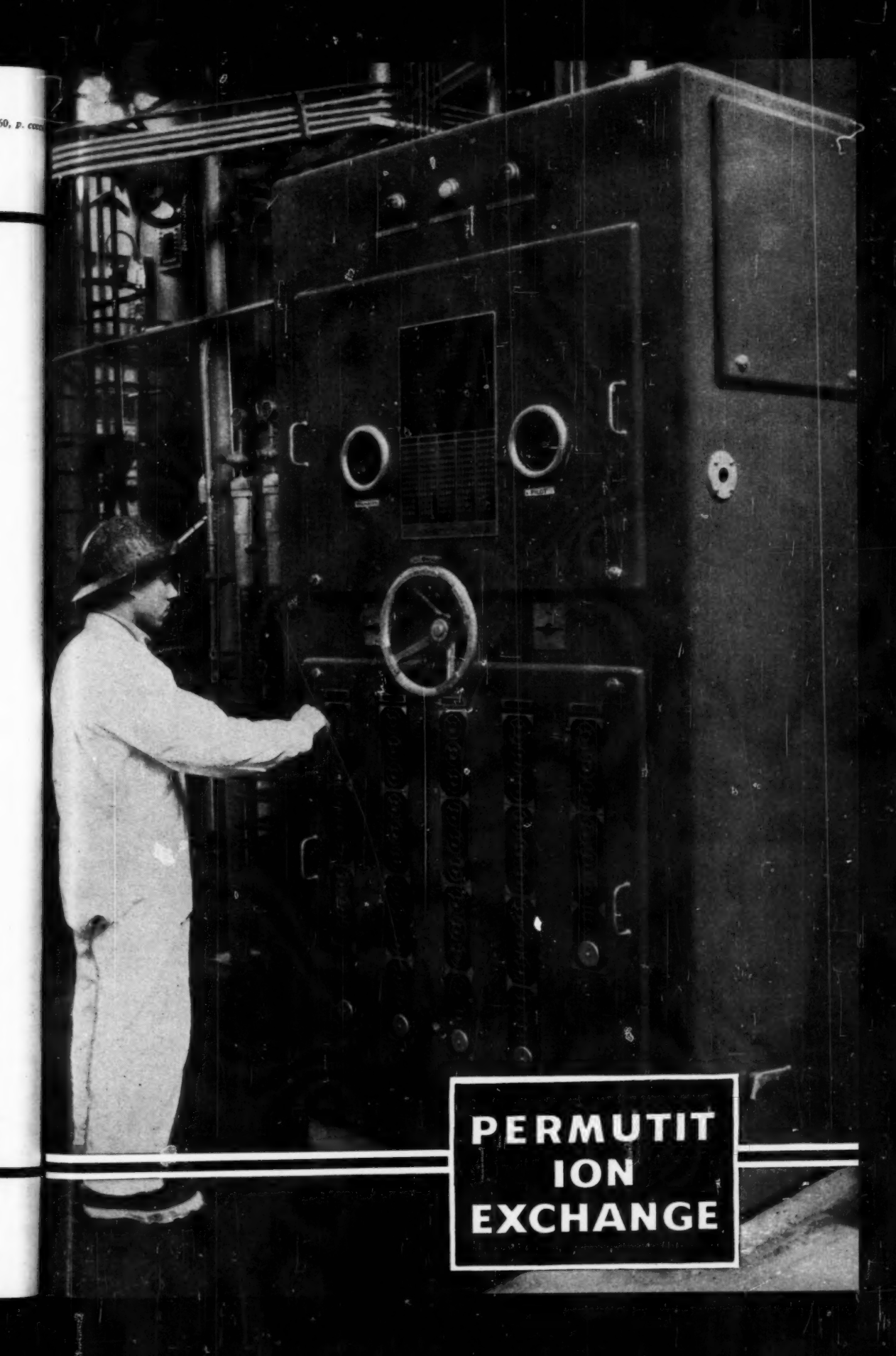
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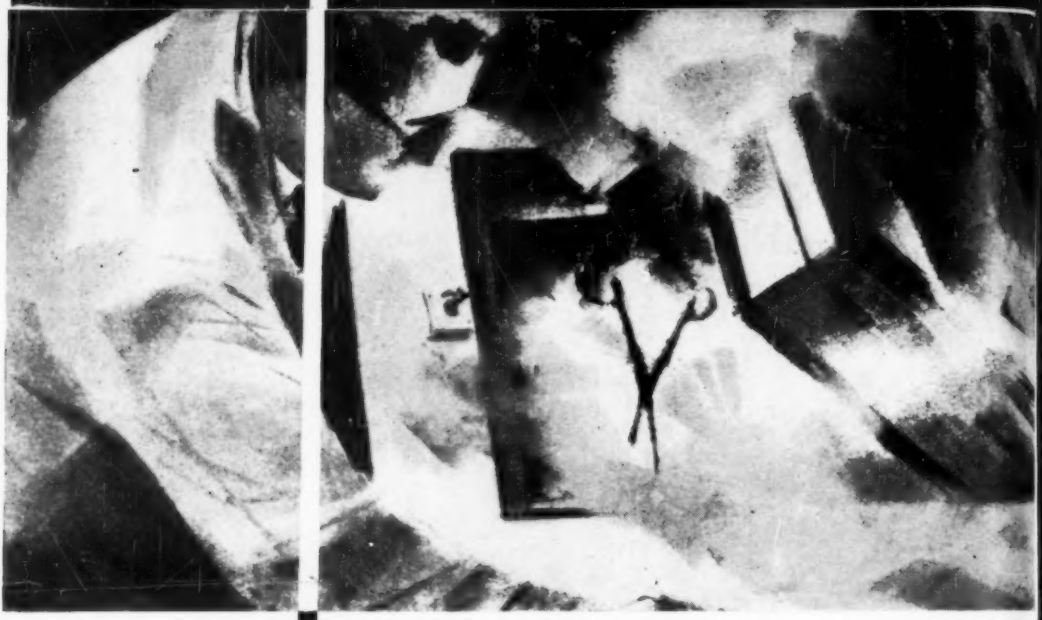

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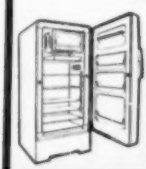

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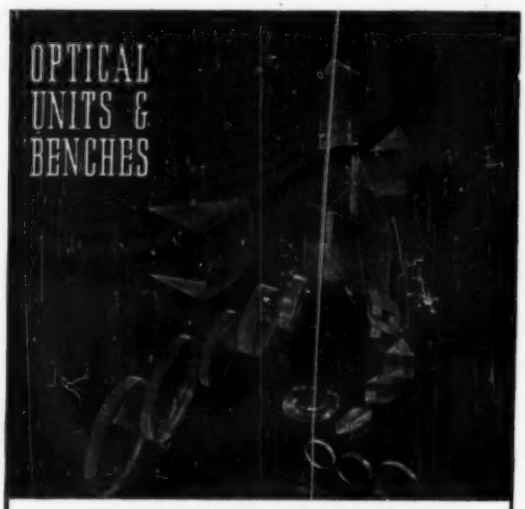

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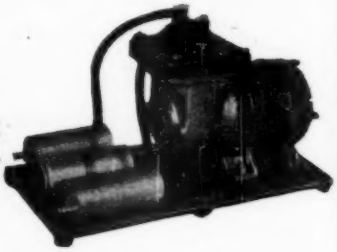

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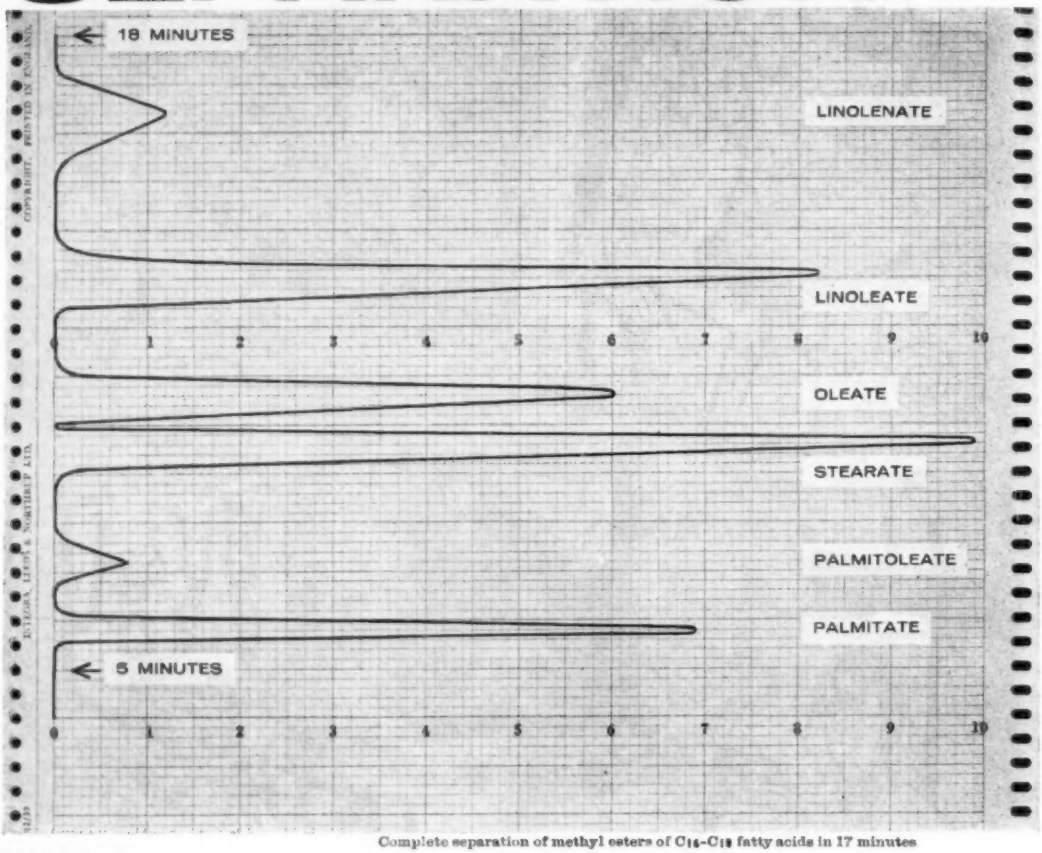

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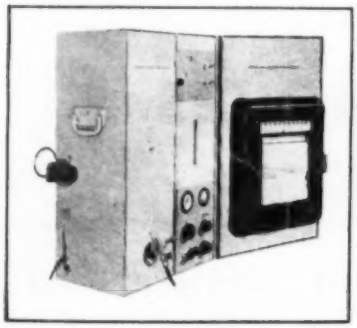

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Professor B. G. Neal

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Professor R. H. Macmillan

Two Lecturers or Assistant Lecturers, one with particular interests in the fields of Heat Transfer and Thermodynamics, the other preferably having experience in the Design of Machines, but applicants with other interests will be considered.

Research Demonstrator to undertake a limited amount of teaching and pursue supervised research preferably in Heat Transfer, Hydrodynamic power transmission, Fatigue or creep.

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Professor W. Fishwick

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Lecturer or Assistant Lecturer with interests in any branch of Electrical Engineering, including Theoretical Electro-technics.

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Salaries are on the following scales, to which are added F.S.S.U. benefits and family allowances. Lecturer, £1,050 by £50 to £1,400 by £75 to £1,850 per annum; Assistant Lecturer, £800 by £50 to £950 per annum; Research Demonstrator, £700 per annum.

Further particulars can be obtained from the Registrar, University College, Singleton Park, Swansea, by whom applications (two copies), must be received not later than Saturday, January 21, 1961.

UNIVERSITY COLLEGE OF RHODESIA AND NYASALAND

Applications are invited for Lectureships or Assistant Lectureships in: GEOLOGY, MATHEMATICS, PHYSICS. Salary scales: Assistant Lecturer, £1,200 by £50 to £1,350 per annum; Lecturer, Grade II, £1,350 by £50 to £1,600 per annum; Grade I, £1,600 by £50 to £1,900 per annum. Passages to Salisbury for appointee, wife, and dependent children on appointment, with allowance for transport of effects. Superannuation and medical aid schemes. Unfurnished accommodation at rent of 15 per cent of salary. Assistance given for triennial visits overseas.

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AUSTRALIA
UNIVERSITY OF NEW SOUTH WALES
VACANCIES FOR STAFF IN SCHOOL OF BIOLOGICAL SCIENCES

The School of Biological Sciences of the University of New South Wales is under the headship of the professor of Biochemistry (Professor B. J. Ralph) and consists of four departments. These are the Department of Biochemistry (Professor B. J. Ralph), Department of Microbiology (Professor to be appointed), Department of Botany (Dr. Mary Hindmarsh) and Department of Zoology (Dr. Elsie M. Stephenson). The undergraduate work of the School is concerned with the preparation of students, at pass and honours levels, in biochemistry, microbiology, botany, zoology and entomology in the Faculty of Science, and biochemistry, microbiology and entomology in the Applied Biology course. In 1962 and later years the relevant Departments will be responsible for medical biochemistry and medical microbiology in the courses in medicine and medical science. Specialized subjects are also provided for courses in the Faculty of Applied Science. All departments of the School are active in research and prepare students for Masters' degrees and for the Doctorate of Philosophy.

Lecturers in the Departments of Botany and Zoology are expected to assist generally with elementary teaching, but are otherwise encouraged to specialize within their own particular discipline. The Departments of Biochemistry and Microbiology are particularly concerned with the application of these sciences, and formal postgraduate courses in industrial biochemistry and microbiology are being developed. As the School handles a considerable number of part-time and full-time students, some evening lecturing is inevitable, but this is limited in extent. Applicants with experience and/or research interests in any of the divisions of botany or zoology, or in medical biochemistry or medical microbiology are particularly sought.

The following vacancies exist: Senior Lecturers, Lecturers: All Departments. Teaching Fellows: Botany, zoology and biochemistry. Senior Tutors: Biochemistry, botany and microbiology. Tutor/Demonstrators: Botany and zoology.

Applicants should possess at least the following academic qualifications: Senior Lecturer, a higher degree or equivalent qualifications. Lecturer, a degree with honours or equivalent qualifications. For these positions postgraduate experience is desirable: Teaching Fellow: Applicants must possess a degree with honours. Teaching Fellows are required to register for a higher degree. Senior Tutor: a degree with teaching experience. Tutor: a degree. Tutors and Senior Tutors will have opportunity to study for a Master's degree on a part-time basis.

Further details may be obtained from the Head of the School of Biological Sciences.

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GOVERNMENT OF THE FEDERATION OF RHODESIA AND NYASALAND
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Regulations governing the award of the Fellowships may be obtained from the Registrar, the University, Manchester 13, to whom applications should be sent not later than February 14, 1961.

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CATHAYS PARK, CARDIFF
DEPARTMENT OF APPLIED PHYSICS

Applications are invited for the posts of (a) Lecturer or Assistant Lecturer (Grade B) in Electronics. (b) Assistant Lecturer (Grade B) in Physics. Applicants should have a good honours degree in physics or electrical engineering, and be able to teach (a) Electronics and/or Telecommunications, (b) Physics and/or Applied Physics, to students taking Dip.Tech. Courses, and have had industrial and/or research experience. Teaching experience at University or Technical College level would be an advantage. Successful candidates would be expected to pursue some research and every assistance and encouragement will be given to this end. The Department's research activities are largely confined to Solid State Physics and application from those particularly interested in some aspect of Solid State Physics or Applied Physics would be particularly welcome. Salary (a) Lecturer, £1,370 by £35 to £1,550 per annum. (b) Assistant Lecturer (Grade B), with good honours degree, £865 by £27 10s. to £1,315 per annum starting salary according to qualifications.

Further particulars and application forms can be obtained from the Registrar at the College, to whom they should be returned by January 14, 1961.

ROBERT E. PRESSWOOD,
Clerk to the Governing Body.

City Hall,
Cardiff.

AUSTRALIA
UNIVERSITY OF NEW SOUTH WALES
SENIOR LECTURER IN PHYSIOLOGY
FACULTY OF MEDICINE

The University invites applications for appointment to the position of Lecturer in the School of Physiology within the Faculty of Medicine. Salary: £A.2,579 range £A.3,029 per annum. Commencing salary according to qualifications and experience. Applicants must have research and teaching experience in the fields of cardiovascular physiology and respiratory physiology. The successful applicant will participate in the new teaching programme of the School and establishment of the cardiopulmonary research laboratory. The appointee will be expected to enter on duty in this position about June, 1961. First-class ship fares to Sydney of the appointee and his family will be paid. Appointee will be eligible, subject to satisfactory medical report, to contribute to the State Superannuation Fund.

Four copies of applications (including the names of two referees) should be lodged with the Agent-General for New South Wales, 56 Strand, London, W.C.2, and a copy forwarded by airmail in an envelope marked "University Appointment" to the Bursar, the University of New South Wales, Box 1, Post Office, Kensington, New South Wales, Australia, before January 31, 1961. Applicants resident outside the United Kingdom and Eire need only submit one copy of their application to the London address.

AGRICULTURAL RESEARCH COUNCIL

Assistant Experimental Officer required, to be responsible for preparative work in a research programme concerned with biochemical and developmental genetics. General experience of biological laboratory methods and a special experience of biochemistry, genetics embryology or histology, an advantage. Salary within scale £490 to £945 according to age. Candidates should have a School Leaving Certificate with passes in three subjects at higher level, including two science subjects; a Higher National Certificate, or equivalent. Superannuation.

Apply, with names of three referees to, the Secretary, Poultry Research Centre, King's Buildings, West Mains Road, Edinburgh, 9, within two weeks.

ESSEX COLLEGE
ASSUMPTION UNIVERSITY OF WINDSOR WINDSOR, ONTARIO, CANADA

The Department of Physics offers Graduate Assistantships to candidates wishing to proceed to an M.Sc. in physics. Stipend up to $2,000 for eight months, depending on qualifications and duties in the department. There is a possibility of additional income during the summer months. At present research is proceeding in beta-ray spectroscopy, optical pumping, molecular collision problems, and electromagnetic interactions in shock waves.

Inquiries should be addressed to the Chairman, Department of Physics, Assumption University of Windsor.

AUSTRALIA
UNIVERSITY OF NEW SOUTH WALES
LECTURER IN FOOD TECHNOLOGY

The University invites applications for appointment to the position of Lecturer in the Department of Food Technology. Salary: £A.1,759 range £A.2,464 per annum. Commencing salary according to qualifications and experience. Applicants should possess a degree with honours in biochemistry or microbiology, or equivalent qualifications, and some previous experience in the food industry is desirable but not essential. The successful applicant will lecture to undergraduate and postgraduate students in food technology and undertake research in some aspects of biochemistry or microbiology, as applied to food science or food technology. First-class ship fares to Sydney of appointee and his family will be paid. Successful applicant will be eligible to contribute to the State Superannuation Fund.

Four copies of applications, including the names of two referees, should be lodged with the Agent-General for New South Wales, 56 Strand, London, W.C.2, and a copy forwarded by airmail in an envelope marked "University Appointment" to the Appointments Section, the University of New South Wales, Box 1, Post Office, Kensington, New South Wales, Australia, before January 27, 1961. Candidates outside the United Kingdom and Ireland need only submit one copy of their applications to the London address.

AUSTRALIA
UNIVERSITY OF NEW SOUTH WALES
FACULTY OF ENGINEERING
TEACHING FELLOWS

Vacancies for teaching fellows exist in the Schools of Mechanical, Electrical and Civil Engineering. Applicants must possess a degree with honours, and will be expected to enrol for a higher degree and undertake a limited amount of teaching duties. Engagement is on an annual basis to a maximum of three years. Salary: £A.1,129 range £A.1,349 per annum. Commencing salary for all positions will be according to qualifications and experience.

Further details regarding these positions may be obtained from the Head of the appropriate school. Ship fares to Sydney of appointees and their families will be paid. Four copies of applications (including the names of two referees), should be lodged with the Agent-General for New South Wales, 56-57 Strand, London, W.C.2, and a copy forwarded by airmail to the Appointments Section, the University of New South Wales, Box 1, Post Office, Kensington, New South Wales, Australia, before January 27, 1961.

UNIVERSITY OF ST. ANDREWS

Applications are invited for a Senior Lectureship or a Lectureship in Electrical Engineering in the Department of Electrical Engineering, Queen's College, Dundee, to commence not later than October 1, 1961. It is intended to appoint the most suitable candidate irrespective of his particular interest. Salary scales: Senior Lectureship, £1,925 by £75 to £2,425; Lectureship, £1,050 by £50 to £1,400 by £75 to £1,850 (efficiency bar £1,400). F.S.S.U.; family allowance, grant towards furniture removal expenses.

Applications (six copies), containing the names of three referees, to the undernoted, from whom further particulars may be obtained, not later than January 21, 1961.

PATRICK CUMMING,
Joint Clerk to the University Court.

Queen's College,
Dundee.

UNIVERSITY COLLEGE OF THE WEST INDIES

Applications are invited for (a) Senior Lectureship and (b) Lectureship in Geology. The Senior Lecturer will be for the time being, the Head of the Department of Geology which is about to be established and which will prepare students for degrees of University of London as from October, 1961. Salary scales (under review) (a) £1,500 by £75 to £2,250 per annum. (b) £1,150 by £50 to £1,450 by £75 to £1,900 per annum. Point of entry determined by qualifications and experience. Child allowance. F.S.S.U. Unfurnished accommodation at rental not in excess of 10 per cent of basic salary. Up to five full passages on appointment, termination and study leave (once every three years). Duties to be assumed by April 1, 1961, or as soon as possible thereafter. The Head of Department will be eligible for entertainment allowance at the rate of £200 per annum.

Detailed applications (six copies), naming three referees, by January 27, 1961, to Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1, from whom further particulars may be obtained.

AUSTRALIA

COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANIZATION

RESEARCH PHYSICIST

The Organization's Division of Metrology, National Standards Laboratory, Sydney, N.S.W., invites applications for the position of Research Physicist in the Interferometry Section from honours graduates, majoring in physics with some years' postgraduate research experience, preferably to Ph.D. level.

The Section, which has a well-equipped vacuum coating laboratory as well as equipment for studying surfaces and thin films by multiple-beam interferometry, is engaged on research and development in interferometry, particularly the measurement of length and associated quantities such as flatness, surface structure and optical phase change at reflection. The appointee is required for research in interference spectroscopy and light source development involving the production study and use of various spectral radiations from isotopic light sources as primary and secondary wavelength standards in metrology.

SALARY: Dependent on qualifications and experience within the ranges: £A.1,510 to £A.2,265 per annum or £A.2,395 to £A.2,720 per annum.

Promotion within C.S.I.R.O. is by merit and may ultimately go beyond the upper limit of the scale within which the original appointment is made.

Fares paid. Further information on conditions etc., supplied on application to:

Mr. E. J. Drake, Chief Scientific Liaison Officer, Australian Scientific Liaison Office, Africa House, Kingsway, London, W.C.2,

to whom applications (quoting Appointment No. 750/222) should be addressed by February 11, 1961.

UNIVERSITY OF EDINBURGH

Applications are invited for I.C.I. Research Fellowships in Biochemistry, Chemistry, Engineering, Pharmacology, Physics or Chemical Technology (Chemical Engineering), normally tenable for a period of three years. Some appointments will date from October 1, 1961 (or earlier in the case of selected candidates who may be available before that date). The initial salary will depend upon qualifications and experience, but will normally be within the range £800 to £1,100 per annum, together with F.S.S.U. benefits and family allowance. In the case of an overseas applicant, the University is also prepared to consider the provision of an allowance towards defraying the cost of travel to and from Great Britain. Forms of application and further particulars may be obtained from the undersigned.

Applications (two copies), should be submitted not later than February 16, 1961.

CHARLES H. STEWART,
Secretary to the University.

UNIVERSITY OF MELBOURNE

SENIOR LECTURESHIP IN CLINICAL BIOCHEMISTRY

Applications are invited for the above-mentioned position. The Senior Lecturer's duties will be chiefly with medical students and it is essential that he should be familiar with modern methods in clinical biochemistry. It is also desirable that he should hold a medical qualification. The salary range is £A.2,480 per annum to £A.2,950 per annum or, if medically qualified, £A.2,600 per annum to £A.3,250 per annum. Commencing salary will be fixed according to qualifications. Superannuation similar to F.S.S.U. in Great Britain will be provided.

Conditions of appointment may be obtained from the Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1. Applications close in Australia and London on February 4, 1961.

UNIVERSITY OF OXFORD

DEPARTMENT OF BIOCHEMISTRY

It is proposed to appoint a Departmental Demonstrator in Biochemistry as soon as possible. The salary will be up to £1,000 per annum (or up to £1,150 in the case of medically qualified candidates). The person appointed will be required to become a member of the Federated Superannuation System for Universities not later than two years after the date of his appointment, and an allowance of £50 per annum for each dependent child is payable.

Three copies of application, including a statement of age, qualifications and experience and giving the names of two referees, should be sent to the Administrator, Department of Biochemistry, South Parks Road, Oxford, not later than March 4, 1961.

FEDERAL GOVERNMENT OF NIGERIA

SCIENTIFIC OFFICER
(MINERAL DRESSER)

The Geological Survey Division of the Federal Ministry of Mines and Power have vacancies for Scientific Officers (Mineral Dressers) to undertake research on mineral dressing, particularly in connection with the recovery of minerals from alluvial concentrates in the Plateau tinfields.

Candidates should hold a good honours degree in mineral dressing or mining engineering, preferably with some experience in industry. Candidates should preferably not be over 35 years of age.

Contract appointment for one tour of 15 to 18 months in the first instance with prospect of extension. Salary according to qualification and experience £1,434 to £2,196 per annum (including inducement addition) plus gratuity of £100 to £150 on satisfactory completion of service. Outfit allowance of £60 payable for salaries not exceeding £1,740. Free passages for officer and wife. Liberal home leave on full pay. Quarters and income tax at low local rates. Children's allowances while separated.

Write for application forms and further particulars, stating briefly age, qualifications and experience, to the Appointments Secretary, Federal Public Service Commission, Nigeria House, 9 Northumberland Avenue, London, W.C.2, quoting J.2/8.

IMPERIAL CANCER RESEARCH FUND

(DIVISION OF BIOCHEMISTRY)

Technical Officer (age 22 or over). Applicants should possess at least Higher National Certificate in Chemistry or equivalent qualifications, and have had experience in synthetic organic chemistry and/or micro-analysis and/or spectroscopy. Salary: £920 by £35 by £40 to £1,260 plus £20 London weighting. Junior Technical Officer (age 18 or over). Applicants should have G.C.E. A-level passes in chemistry and physics or equivalent. Training will be given in methods of micro-analysis and spectroscopy. Salary: £410 by £35 by £40 to £860 plus £10 London weighting.

Applications should be addressed to: Scientific Administrator, Imperial Cancer Research Fund, Lincoln's Inn Fields, London, W.C.2.

UNIVERSITY OF LIVERPOOL

Applications are invited for Imperial Chemical Industries Research Fellowships in Physics, Chemistry, Biochemistry, Engineering, Metallurgy and Pharmacology or any related subjects. Appointments will date from October 1, 1961. The salary will depend upon qualifications and experience, but will normally be within the range £700 to £1,000 per annum, together with F.S.S.U. benefits and family allowances.

Applications, three copies, stating age, details of qualifications and experience, publications, and research work in progress and completed, together with the names of two referees, should be received not later than February 11, 1961, by the Registrar, from whom further particulars may be obtained. (Candidates overseas who find it more convenient to do so may send one copy only by airmail.) Ref. CV/N.

FEDERAL GOVERNMENT OF NIGERIA

SCIENTIFIC OFFICER
(GEOPHYSICIST)

The Geological Survey Division of the Federal Ministry of Mines and Power have vacancies for Scientific Officers (Geophysicist) to conduct geophysical surveys using a.c. and d.c. resistivity apparatus, d.c. potential equipment, vertical forces magnetic variometer, shallow seismic gear, and equipment for logging resistivities and temperature gradients in boreholes.

Candidates must hold a good honours degree in geophysics, physics or engineering. Alternatively a good honours degree in geology, with physics or applied mathematics as subsidiary subjects. Experience in applied geophysics and some knowledge of electrical circuitry and an ability to maintain apparatus will be added advantages. Candidates should preferably not be over 35 years of age.

Contract: appointment for one tour of 15 to 18 months in the first instance with prospect of extension. Salary according to qualifications and experience £1,434 to £2,196 per annum (including inducement addition) plus gratuity of £100 to £150 on satisfactory completion of service. Outfit allowance of £60 payable for salaries not exceeding £1,740. Free passages for officer and wife. Liberal home leave on full pay. Quarters and income tax at low local rates. Children's allowances while separated.

Write for application forms and further particulars, stating briefly age, qualifications and experience, to the Appointments Secretary, Federal Public Service Commission, Nigeria House, 9 Northumberland Avenue, London, W.C.2, quoting J.3/8.

WEST AFRICAN COCOA RESEARCH INSTITUTE

GHANA
SENIOR BOTANIST

To take charge of the Botany Division comprising plant breeding and plant physiology sections. Candidates must have a first- or second-class honours degree in botany or equivalent qualification, with at least 15 years' research experience and a good record of published work. Proven ability to plan and supervise research programmes and experience with tree crops, preferably in the tropics, is also desirable. Appointment on contract/gratuity terms for two tours each of 15 to 18 months in the first instance. Salary in scale £2,460 to £2,940. Gratuity £12 10s. for each month of satisfactory service. Quarters with heavy furniture provided at moderate rental. Free return passages once in each tour for officer, wife and up to three children under 18 years of age. Generous leave. Education allowances.

Apply, stating name in full and giving brief particulars of qualifications and experience, to Director of Recruitment, Colonial Office, S.W.1, quoting BCD.197/200/028/C1.

JUNIOR TECHNICIAN FOR BIOCHEMICAL work required at once at Twyford Laboratories Ltd., Twyford Abbey Road, London, N.W.10. Training given in general laboratory and workshop practice, leading to assisting in research projects. Best of conditions and prospects. G.C.E. advanced level in two science subjects or equivalent required.—Apply to Secretary at above address.

UNIVERSITY COLLEGE OF THE WEST INDIES

Applications are invited for (a) Senior Lectureship or (b) Lectureship in Mathematics in the Faculty of Engineering, Trinidad. Interest in the application of mathematics to engineering essential. Salary scales (under review) (a) £1,500 by £75 to £2,250 per annum, (b) £1,150 by £50 to £1,450 by £75 to £1,900 per annum. Entry point determined by qualifications and experience. Child allowance. F.S.S.U. Unfurnished accommodation at rental not in excess of 10 per cent of basic salary. Up to five full passages on appointment, termination and study leave (once every three years). Duties to be assumed by June 1961.

Detailed applications (six copies), naming three referees, by January 25, 1961, to Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1, from whom further particulars may be obtained.

SCUNTHORPE HOSPITAL MANAGEMENT COMMITTEE

LINCOLNSHIRE RADIOTHERAPY CENTRE

PHYSICS LABORATORY

Applications invited for post of Health Physics Technician to carry out radiation and contamination measurements involved in use of X-rays, radium and isotopes by the staffs of the Lincolnshire Hospitals. The Radiotherapy Centre now at Scunthorpe will be transferred to St. George's Hospital, Lincoln during 1961. Educational requirements O.N.C. in Applied Physics, Mechanical or Electrical Engineering, Inter.B.Sc., City and Guilds Intermediate of equivalent qualification or completion of engineering or appropriate apprenticeship. Minimum age 21. Salary scale £570 by £20 (2) by £25 (4) to £710.

Form of application from Hospital Secretary, War Memorial Hospital, Scunthorpe.

UNIVERSITY OF QUEENSLAND

SENIOR LECTURER OR LECTURER IN PHYSICAL CHEMISTRY

Applications are invited for appointment to the above-mentioned position dependent upon qualifications. Applicants should have a higher degree preferably a doctorate with special qualifications in some branch of physical chemistry. In the case of the senior lectureship university and research experience is essential. The appointee's duties will include lecturing to undergraduate and honours students and he will be expected to engage in research and direct the research of a limited number of students. Salary range £A.2,520 by £A.70 to £A.2,870 per annum for Senior Lecturer and £A.1,830 by £A.70 to £A.2,330 per annum for Lecturer. The successful applicant will enjoy the privileges of superannuation, study leave, travel grants and other benefits available to the academic staff.

Further particulars may be obtained from the Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1. Applications close, in Australia and London, on February 15, 1961.

UNIVERSITY OF QUEENSLAND

LECTURER IN PROTOZOOLOGY

Applications are invited for the above-mentioned post within the Department of Parasitology. Applicants should have a Ph.D. or a degree in veterinary science with postgraduate experience in protozoology. Duties will be primarily teaching and research on the parasitic protozoa within the Faculties of Science and Veterinary Science, but it is desirable that applicants should also have knowledge and experience of parasitic arthropods particularly those of veterinary importance. Salary range £A.1,830 by £A.70 to £A.2,330. The successful applicant will enjoy the privileges of superannuation, study leave, travel grants and other benefits available to the academic staff.

Further particulars may be obtained from the Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1. Applications close, in Australia and London, on February 28, 1961.

ELECTRONICS MECHANIC WITH LIGHT fitting and turning experience required to build and maintain electronic and instrumentation devices for agricultural engineering research and assist experimental work. Time served or ex-Service tradesman would suit. City and Guilds Certificates or National Certificate desirable. Superannuated staff post. Grade Senior Assistant (Scientific). Minimum age 25. Salary £755 to £1,010.—Further particulars from Secretary, N.I.A.E., Scottish Station, Howden, Mid Calder, Midlothian.

UNIVERSITY OF QUEENSLAND

SENIOR LECTURER IN GEOLOGY (GEOCHEMIST)

Applications are invited for the above-mentioned post, which is a responsible teaching position with excellent opportunity for geochemical and petrological research in a large university. Applicants should have a science degree and research experience to Ph.D. or comparable level; present dominant interest should be in geochemistry (theoretical and economic). Some experience in rock and mineral analysis and ability to do fundamental research is expected. An interest in isotope geology and at least some experience in mass spectrometry is desirable. Some interest in X-ray spectrography or other special techniques could be an advantage. Salary £A.2,520 by £A.70 to £A.2,870 per annum. The successful applicant will enjoy the privileges of superannuation, study leave, travel grants and other benefits available to the academic staff.

Further particulars may be obtained from the Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1. Applications close, in Australia and London, on March 31, 1961.

UNIVERSITY OF LEEDS

ELECTRONIC COMPUTING LABORATORY

Applications are invited for appointment as Lecturer in the Electronic Computing Laboratory at a salary on the scale £1,050 by £50 to £1,400 by £75 to £1,850 (efficiency bar at £1,550) a year according to age, qualifications and experience. Previous experience in the application of computers in statistics and/or operational research desirable but not essential.

Applications (three copies), stating date of birth, qualifications and experience, together with the names of three referees, should reach the Registrar, the University, Leeds, 2 (from whom further particulars may be obtained) not later than January 20, 1961. Applicants from overseas may apply in the first instance by cable naming three referees, preferably in the British Isles.

UNIVERSITY OF MANCHESTER

Applications are invited for the following posts in the Department of Physics of the Faculty of Science: Lecturer in Physics (Experimental); Lecturer in Theoretical Physics; Assistant Lecturers in Physics. The research interests of the Department include Nuclear and High-Energy Physics, Optics, Low Temperature and Molecular Physics. Duties to commence as soon as possible. Salary scales: Lecturer, £1,050 to £1,850 per annum, initial salary according to qualifications and experience; Assistant Lecturer, £800 to £950 per annum. Membership of F.S.S.U. and Children's Allowance Scheme.

Applications must be sent not later than January 14, 1961, to the Registrar, the University, Manchester 13, from whom further particulars and forms of application may be obtained. Applications by airmail letter in the first instance will be received from overseas candidates, who should state the names and addresses of at least three persons to whom reference can be made.

THE BOREAL INSTITUTE OF THE University of Alberta invites applications for the position of Executive Director. The Executive Director will be a full-time appointee who will aid the Directorate in planning the activities of the Institute, implement the decisions of the Directorate, administer the daily operations of the Institute, solicit funds for the advancement of the Institute, and publicize the activities and accomplishments of the Institute. Applicants must have an interest in the North, administrative ability, ability to deal with the public, and fluency in written and spoken English. Applicants should be willing and able to travel in the North and should have some experience in the North. Applications must include a complete résumé of experience, educational background, references, other pertinent data, and a recent photograph. The appointment will be effective April 1, 1961. Salary is dependent upon qualifications, but will be in the range $8,000 to $12,000 a year.—Applications should be submitted to reach the Boreal Institute, University of Alberta, Edmonton, Alberta, Canada, prior to February 1, 1961. Applications will be held in confidence if desired.

SCIENCE ASSISTANT (FEMALE) REquired for the Public Health Laboratory, Portsmouth. Graduates with microbiology as one subject and an interest in virology preferred. Salary according to experience.—Applications, giving age and qualifications to the Director, Public Health Laboratory, Priorsdean Hospital, Milton Road, Portsmouth.

WOOL INDUSTRIES RESEARCH ASSOCIATION

A vacancy occurs for a Mathematician with special interest in applied mathematics or theoretical physics, lively mind, and interest in investigating wide variety of problems (mechanical, physical, numerical, etc., in nature) arising in textile research. Ph.D. or previous research experience desirable. Starting salary up to £1,590 per annum (with F.S.S.U. benefits), according to qualifications and experience.

Apply to the Association, Torridon, Headingley Lane, Leeds, 6.

UNIVERSITY OF CANTERBURY

CHRISTCHURCH, NEW ZEALAND

LECTURER IN CHEMISTRY

Applications are invited for the above-mentioned post in the Department of Chemistry. Applicants should have special qualifications or experience in Inorganic or Physical Chemistry. The salary attached to the position will be at the rate of £1,250 per annum, rising by annual increments of £75, to £1,700 per annum. Commencing salary will be in accordance with qualifications and experience. Approved fares to Christchurch will be allowed for the appointee, his wife and children. In addition actual removal expenses will be allowed within certain limits.

Further particulars and information as to the method of application may be obtained from the Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1. Applications close in New Zealand and London on February 17, 1961.

QUEEN'S UNIVERSITY OF BELFAST

The Senate of the Queen's University of Belfast invites applications for a Senior Lectureship in charge of the Department of Engineering Mathematics from October 1, 1961. The Department is responsible for teaching mathematics to engineering students throughout the University and candidates should preferably have a research interest in the application of mathematics to some engineering subject. Salary range £1,700 to £2,275 plus contributory pension rights under the F.S.S.U. Initial placing on the salary scale will depend on qualifications and experience. Applications should be received by January 30, 1961.

Further particulars may be obtained from G. R. Cowie, M.A., LL.B., J.P., Secretary to the University.

UNIVERSITY OF WESTERN AUSTRALIA

LECTURESHIP IN GEOGRAPHY

Applications are invited for appointment to the above-named position in the Faculty of Economics. Preference will be given to candidates with training and/or publications in the field of human and economic geography. The salary range for the position is £A.1,750 by £A.75 to £A.2,350 per annum with superannuation similar to F.S.S.U. Candidates are requested to state whether they have specialized in any regional field and, if so, to specify the area. The appointee will be expected to take up duty as early as possible in 1961.

It is important that intending applicants obtain details of the procedure to be followed in applying for the post and a copy of the Conditions of Appointment, before submitting their applications. This information is available from the Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1. Applications close, in Australia and London, on February 25, 1961.

BIRKBECK COLLEGE

(UNIVERSITY OF LONDON)

Applications are invited for the post of Assistant Lecturer in Physics. Salary scale £800 by £50 to £950 per annum, plus London allowance of £60 per annum.

Further particulars should be obtained from the Secretary, Birkbeck College, Malet Street, W.C.1. Closing date for applications: January 31, 1961.

ESSEX COLLEGE

ASSUMPTION UNIVERSITY OF WINDSOR WINDSOR, ONTARIO, CANADA

The Department of Mathematics offers Graduate Assistantships to candidates wishing to proceed to the M.Sc. in Mathematics. Stipend up to $2,000 for eight months depending on qualifications and duties in that Department. Assistants may pursue courses in pure or applied mathematics.

Inquiries should be addressed to the Head of the Department of Mathematics.

UNIVERSITY OF ST. ANDREWS

Applications are invited for a Lectureship in Midwifery in the Department of Midwifery and Gynaecology, Queen's College, Dundee. Applicants must have had a special training in physiology and must be prepared to take a special interest in the physiology of human reproduction. Salary scale, either clinical grade: £1,050 by £100 to £2,350 (efficiency bar £1,550), or non-medical science scale: £1,050 by £50 to £1,400 by £75 to £1,850 (efficiency bar £1,400). F.S.S.U.; family allowance; grant towards furniture removal expenses.

Applications (six copies) containing the names of three referees to the undernoted, not later than January 21, 1961.

PATRICK CUMMING,
Joint Clerk to the University Court.
Queen's College, Dundee.

ESSEX COLLEGE

ASSUMPTION UNIVERSITY OF WINDSOR
WINDSOR, ONTARIO, CANADA

The Department of Physics invites applications for the following appointments: two Experimental Physicists, one Theoretical Physicist. The successful applicants will be expected to instruct undergraduate and graduate students and devote a part of their time to research. Preference will be given to candidates with interests in low energy nuclear physics, solid state physics, or molecular spectroscopy. The rank and salary will depend upon qualifications and experience, but the salary will not be less than $7,000 at Ph.D. level.

Inquiries should be addressed to the Chairman, Department of Physics, Assumption University of Windsor.

NORTHAMPTON COLLEGE OF ADVANCED TECHNOLOGY

LONDON

READER IN APPLIED NUCLEAR PHYSICS

The Governing Body invites applications for the above appointment in the Department of Applied Physics. Applicants should possess high academic qualifications and considerable research experience in this branch of applied physics. Salary scale: £1,836 rising to £2,148 per annum.

Further particulars and a form of application can be obtained from the Secretary, Northampton College of Advanced Technology, St. John St., London, E.C.1.

ST. MARY'S HOSPITAL

PADDINGTON, W.2

Chemist required for the post of Research Assistant in the Department of Chemical Pathology. The work is suitable for a higher degree and will be on problems associated with steroid metabolism. Previous experience an advantage. Salary up to £750 per annum, the post being for one year in the first instance.

Applications, giving full details of age, qualifications and experience, together with the names of two referees, to Alan Powditch, House Governor.

MCMASTER UNIVERSITY

POSTDOCTORAL FELLOWSHIPS IN CHEMISTRY

Applications are invited for Postdoctoral Fellowships, National Research Council of Canada and others, tenable in the Department of Chemistry. The N.R.C. awards are for research only, while the other fellowships may also provide some opportunity for teaching. Awards are worth $3,700 to $4,500 per annum, together with travel allowances.

Applications, with curriculum vitae and names of two referees, should be sent promptly to D. B. MacLean, Chairman, Department of Chemistry, McMaster University, Hamilton, Ontario, Canada.

ROYAL VETERINARY COLLEGE REquires Senior Laboratory Technician with knowledge of chemistry. Salary scale £690 by £25 to £815 per annum, plus London weighting. Qualification allowance payable additionally in certain circumstances. Superannuation scheme.—Applications, with full particulars of age and experience, to Assistant Secretary, Royal Veterinary College, Royal College Street, London, N.W.1.

APPLICATIONS ARE INVITED FOR A one-year appointment as Lecturer in Chemical Engineering.—For further information, write Head, Department of Chemistry and Chemical Engineering, University of Saskatchewan, Saskatoon, Saskatchewan, Canada.

UNIVERSITY OF BIRMINGHAM

DEPARTMENT OF GEOLOGY

Research Associate required for work upon hydrated alumino-silicate glasses and melts. Applicants should be graduates with qualifications in mineralogy and petrology or in physical-chemistry or in physics. The appointment will extend until September 30, 1962, as at present determined. Salary in the range £800 by £50 to £850.

Applications, stating age, qualifications and experience, together with the names of two referees, should be sent by January 11, 1961, to Professor F. W. Shotton, University of Birmingham, Edgbaston, Birmingham, 15.

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Apply to: Professor Harry E. Gunning, Head, Department of Chemistry, University of Alberta, Edmonton, Alberta, Canada.

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Apply, naming two referees, to Secretary, N.I.R.D., Shinfield, Reading. Quote ref. 60/15.

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For further information write: Professor H. Grayson-Smith, Head, Department of Physics, University of Alberta, Edmonton, Canada.

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LEICESTERSHIRE

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Application forms or further information from Academic Registrar, quoting Ref. 25/AF.

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KYOTO UNIVERSITY, KYOTO, JAPAN
(Director: Professor Hideki Yukawa)

Applications are invited for a Research Fellow in Theoretical Physics; Nuclear Physics or Solid State Physics (fields covered by the papers which appear in "Progress of Theoretical Physics").

Further particulars and information as to the method of application may be obtained from secretary, Research Institute for Fundamental Physics, Kyoto University, Kyoto, Japan.

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Applications and inquiries to Group Secretary or Consultant Pathologist, giving names of two referees.

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Further information about the course and forms of application, which must be completed and returned not later than Saturday, March 4, 1961, may be obtained from the Warden, the College of Aeronautics, Cranfield, Bletchley, Bucks.

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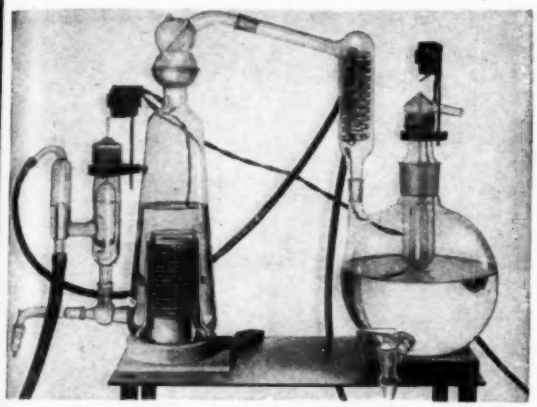

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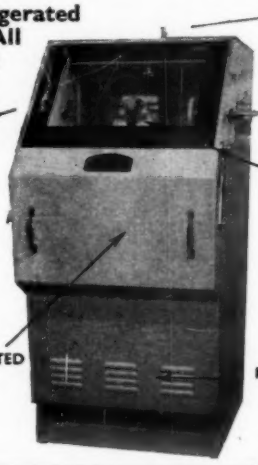

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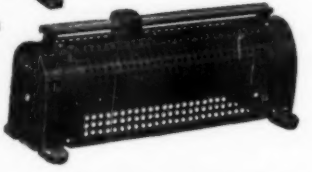

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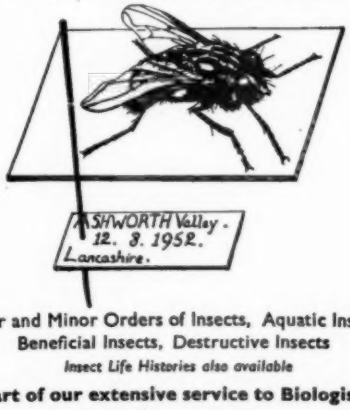

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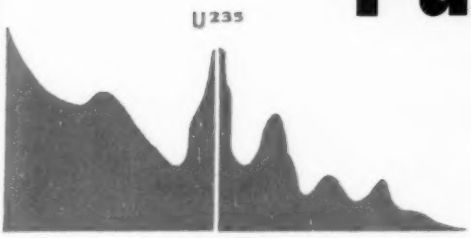

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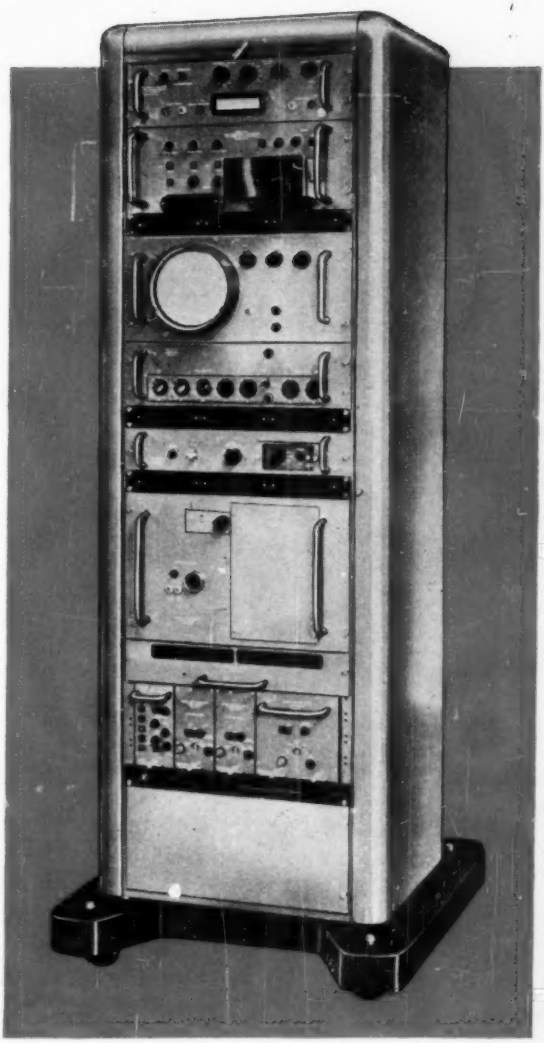

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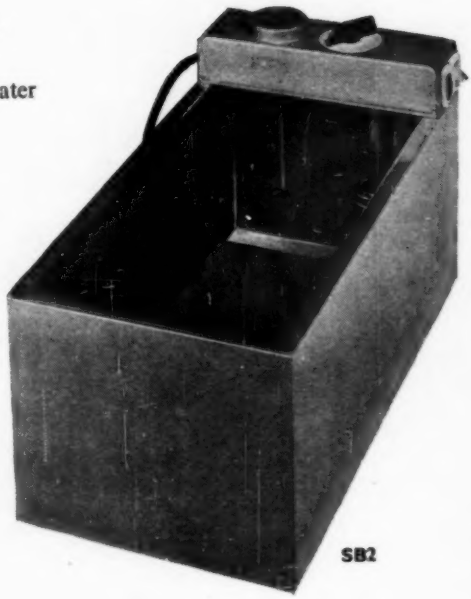

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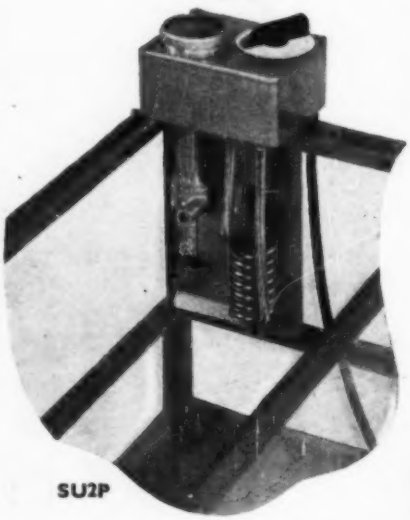

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